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NERVOUS SYSTEM DIFFERENCES BETWEEN THE SEXES AND ACROSS THE MENSTRUAL CYCLE

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NERVOUS SYSTEM DIFFERENCES BETWEEN THE SEXES AND ACROSS THE MENSTRUAL CYCLE

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NERVOUS SYSTEM DIFFERENCES BETWEEN THE SEXES AND ACROSS THE MENSTRUAL CYCLE

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Sex hormones have in vitro effects on the nervous system. Furthermore, the effects of estradiol and progesterone metabolites have neurologic responses on the motor system, evidenced by transcranial magnetic stimulation studies. Sex hormone effects on the nervous system may underlie some of the sex discrepancies seen in athletic performance, injury and cardiovascular events. Investigating sex differences is often complicated by hormonal oscillations across the menstrual cycle; therefore, the aims of this research were to investigate sex and menstrual cycle effects on the motor and cardiovascular systems and the interaction of these two systems. In study 1, motor unit (MU) recruitment patterns of the vastus medialis (VM) and vastus medialis oblique (VMO) were examined in males and females at five menstrual cycle phases. Initial discharge rate between the VM and VMO were different only in females. This VM/VMO discharge discrepancy was only evident in females during the ovulatory and mid luteal menstrual phases. Study 2 examined the frequency domain relationship of the VM and VMO MUs between the sexes and across the menstrual cycle. Males have 256% to 741% greater odds of having coherent MU oscillations in the common drive band than females, indicating a greater common rate modulation. Further evidence indicated MU pairs from the VMO and VM/VMO have 228% and 212% greater odds of having beta

band oscillations than the VM, indicating control of those muscle groups has a common cortical modulation. Study 3 looked at changes in the autonomic nervous system across the menstrual cycle via heart rate variability analysis. Heart rate variability decreases from the follicular to the luteal phases of the menstrual cycle, indicating a decrease in parasympathetic control. In study 4, the time and frequency domain relationship between electrocardiogram and MU discharge timing was examined between the sexes and across the menstrual cycle. The time domain relationship indicated that both males and females have MU time lag centered between 20-25 milliseconds, with an apparent modulation of this relationship across the menstrual cycle. The findings from this series of studies indicate that there are differences between the sexes which are often modified by the menstrual cycle in females.

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Chapter 1: Introduction

Fertility and reproductive function in females is controlled by the hypothalamic-pituitary-gonadal axis, resulting in large and predictable oscillations of estradiol, progesterone, luteinizing hormone and follicle stimulating hormone across the menstrual cycle. Sex steroid oscillations at the plasma level affect the central and autonomic nervous systems because steroids can easily traverse the blood-brain barrier due to their high lipid solubility (Stoffel-Wagner, 2001). Studies in animal models have shown that estradiol has net excitatory effects (Smith et al., 1989; Woolley et al., 1997; Schultz et al., 2009) and that progesterone and its metabolites have net inhibitory effects (Callachan et al., 1987; Smith et al., 1987; Smith et al., 1989; Melchior & Ritzmann, 1994; Frye et al., 2004) on the nervous system. The in vivo effects of sex hormones on discrete portions of the nervous system, as well as the interaction of discrete systems, are needed to fully elucidate how sex and changes in sex hormones alter human biology.

The effects of estradiol and progesterone metabolites on the motor system have been demonstrated in humans using transcranial magnetic stimulation (Smith et al., 1999; Herzog et al., 2001; Smith et al., 2002; Inghilleri et al., 2004). Higher levels of GABA at cortical and subcortical areas in the early follicular phase of the menstrual cycle have been attributed to the lack of estradiol (Epperson et al., 2002; Harada et al., 2011), which inhibits the release of GABA (Schultz et al., 2009). Lower levels of cortical inhibition occur in the late follicular phase compared to the early follicular and mid luteal phases (Smith et al., 1999; Smith et al., 2002), demonstrating the excitatory effects of estradiol on the corticospinal tract. Transcranial magnetic stimulation stimulates the recruitment of motor units via the corticospinal tract (Bawa & Lemon, 1993), but failing to account for

voluntary coordination of movements may discount the effects of sex and sex hormones on real-life movement generation.

Determining if drive to synergistic muscles is altered equally across the phases of the menstrual cycle is important both at the time of motor unit recruitment and maintenance of force. Signal coherence examines the rhythmic frequency behavior of motor units. Coherence between two bioelectric signals indicates that they have a common oscillating neuronal origin (Farmer et al., 1993; Baker et al., 1997). Differential results in motor unit coherence across synergistic muscles during different menstrual phases will demonstrate that neuronal interconnections are modulated by changes in sex hormones in humans.

The changes in GABA at the subcortical level (Harada et al., 2011) indicate that the menstrual cycle sex hormones may also have effects on the autonomic nervous system (ANS). Changes in ANS function across the menstrual cycle have been examined by non-invasive (heart rate variability (HRV)) and invasive (microneurography) methods. Studies using microneurography have shown higher muscle sympathetic nerve activity at the mid luteal phase than at the early follicular phase at rest (Minson et al., 2000; Park & Middlekauff, 2009; Middlekauff et al., 2012) and during an orthostatic challenge (Carter et al., 2009; Fu et al., 2009). This indicates that high levels of female sex hormones can increase sympathetic nerve activity. HRV is an effective measure of parasympathetic activity and early studies suggest that the luteal menstrual phases have lower parasympathetic activity than the follicular phases (Mckinley et al., 2009). Therefore, it is probable that the ANS is modified across the menstrual cycle.

Finally, the menstrual cycle may alter the way in which the motor nervous system and ANS interact. Stimulating the thalamus, subthalamic nucleus and substantia nigra at frequencies higher than 90 Hz results in increases in heart rate, mean arterial pressure and

facilitates leg movement in awake Parkinson's patients (Thornton et al., 2002). This demonstrates that these brain areas can modulate locomotion and the cardiovascular system in parallel. The signal coherence analysis previously described for motor activity has never been applied to two distinct nervous systems; this technique should allow us to determine if there is an interconnection between the cardiac and skeletal muscle. The periaqueductal grey, which facilitates movement and autonomic changes, has been shown to generate signal power in the 60-90 Hz range when measured with intracerebral electrodes (Green et al., 2007). Therefore, we expect coherence between the motor and autonomic systems will be observed in this band. The time-domain relationship between the discharge of cardiac musculature (QRS complex) and the discharge of motor units will also be investigated to determine if the two events have a temporal relationship modified across the menstrual cycle. The interaction of these two separate systems may be defacilitated by increased progesterone metabolite-mediated inhibition.

This dissertation proposes four studies which will examine the neurophysiologic effect of the menstrual cycle on the motor nervous system, autonomic nervous system and the interconnection of these two systems.

Specific Aim 1: Determine the effect of the menstrual cycle on VM and VMO motor unit recruitment properties.

Hypothesis: Initial motor unit firing rate and recruitment threshold will be different between the VM and VMO in females but not males. Differences in initial motor unit firing rate and recruitment threshold between the VM and VMO will be observed in the ovulatory and luteal phases.

Specific Aim 2: Determine if VM and VMO motor unit coherence are modulated differently and if MU coherence is altered across the menstrual cycle during a sustained submaximal isometric task.

Hypothesis: Males will have higher levels of VM and VMO coherence in the common drive and beta bands compared to females. The VM and VMO will exhibit lower levels of coherence in the common drive and beta bands during the ovulatory and luteal phases of the menstrual cycle when progesterone levels are high.

Specific Aim 3: Determine if there are changes in seated resting heart rate variability across the menstrual cycle by using a novel ANCOVA statistical approach which accounts for breathing rate.

Hypothesis: Time domain variables of heart rate variability will be lower in the ovulatory and luteal phases. Frequency domain variables of heart rate variability will also reflect lower parasympathetic activity in the ovulatory and luteal phases with decreases in the high frequency spectral power.

Specific Aim 4: Determine if entrainment between motor unit and cardiac depolarization in the frequency and time-domains are different between the sexes and altered by the menstrual cycle during sustained isometric contraction.

Hypothesis: Significant coherence will be observed between the two systems in the 60-90 Hz frequency ranges for both sexes. This coherence will be lowest during the ovulatory and mid luteal phases due to increased neuronal inhibition from progesterone. Time-domain analyses will indicates that motor drive precedes cardiovascular changes by a predictable time lag.

The overall goal of the research is to examine the importance of sex hormones on central nervous system control of muscle and interaction with the autonomic nervous system. This dissertation will be the first series of studies which examine the effect of the menstrual cycle on motor unit discharge patterns. It is also the first to investigate the

menstrual cycle effect on synergistic muscles controlling patellar tracking. Finally, this will be the first study to examine the coherence of separate nervous systems and determine whether it is affected by the menstrual cycle. This research brings together multiple fields of study to further our understanding of how the nervous systems are influenced by the neuroendocrine system.

REVIEW OF RELATED LITERATURE

Agents altering the fundamental way in which neurons interact can have far reaching effects on seemingly independent systems within the human body. Specifically, the sex hormones which oscillate across the menstrual cycle are known to modify the action of GABA and glutamate, neurotransmitters enabling virtually all communication within the central and peripheral nervous systems. This review will encompass the effect of sex hormones and their effect on neurotransmitter function, their effects on the corticospinal tract in vivo, the assessment of neuronal connections between motor units, neuronal connections between the motor and autonomic systems, and the use of heart rate variability to assess autonomic function.

Hormonal Changes across the Menstrual Cycle

Hypothalamic-Pituitary-Gonadal Axis

The control of adult fertility and reproductive function is controlled by an extensive system called the hypothalamic-pituitary-gonadal axis (HPGA), which includes gonadotropin-releasing hormone (GnRH) cells in the hypothalamus which stimulate the secretion of lutenizing hormone (LH) and follicle-stimulating hormone (FSH) in the anterior pituitary (Yen et al., 1999). In the early part of the menstrual cycle, LH pulses at hourly intervals which stimulate early follicular development and estradiol secretion (Borer, 2003). The rising estradiol levels cause an up-regulation of the activity of GnRH

receptors, which creates a positive feedback effect increasing estradiol and LH and FSH production, precipitating ovulation (Borer, 2003). After the ovum has been discharged, the granulosa and theca cells of the ruptured follicle become the corpus luteum, an organ which synthesizes large quantities of progesterone as well as moderate amounts of estradiol (Yen et al., 1999). The rupturing of the follicle at ovulation marks the end of the follicular phase of the menstrual cycle and the creation of the corpus luteum marks the start of the luteal phase. If pregnancy does not occur, the corpus lutem degenerates and causes a sudden decrease in plasma progesterone and estradiol concentrations (Yen et al., 1999). The cascading events resulting from this substantial decrease in estradiol and progesterone cause a development of new follicles and menstrual bleeding. Thus, in relative terms, the plasma concentrations of LH, FSH, estradiol and progesterone across the normal menstrual cycle are as follows (adapted from Massafra et al., 2000):

	Early Follicular	Late Follicular	Ovulation	Mid Luteal	Late Luteal
FSH	Moderate	Moderate	High	Low	Moderate
LH	Low	Moderate	High	Low	Low
Estradiol	Low	High	High/Moderate	Moderate	Moderate
Progesterone	Low	Low	Moderate	High	Moderate

Table 1.1. Relative Sex Hormone Levels

Menstrual phase can be determined non-invasively via the basal body temperature (BBT) mapping technique. The ovulatory phase is defined as the first day in a sustained temperature rise after basal body temperature nadir (de Mouzon et al., 1984). The BBT has a low precision in predicting exact ovulation date (Guida et al., 1999; Ecochard et al., 2001); however, it is effective in determining the length of the follicular and luteal phases

as well as total cycle length and is used extensively in clinical settings (Barron & Fehring, 2005).

Neurosteroids and their Effects

The term neurosteroid reflects both the action and the origin of the steroid compound, meaning the neurosteroid exerts its action at the brain level and oftentimes are synthesized de novo in the central nervous system (Stoffel-Wagner, 2001). It is presumed that gross sex steroid oscillations at the plasma-level are reflected in the central nervous system because steroids can easily traverse the blood-brain barrier due to their high lipid solubility (Stoffel-Wagner, 2001). The de novo synthesis and metabolism in the brain results in a complex interaction of sex hormones, even before the action of the hormones and their metabolites are considered (see Figure 1.1, Mellon & Griffin, 2002). Nonetheless, oscillating sex hormones and their first and second-order metabolites have been demonstrated to be strong modulators of both inhibitory and excitatory actions and must be discussed when considering various systems' interactions across the menstrual cycle.

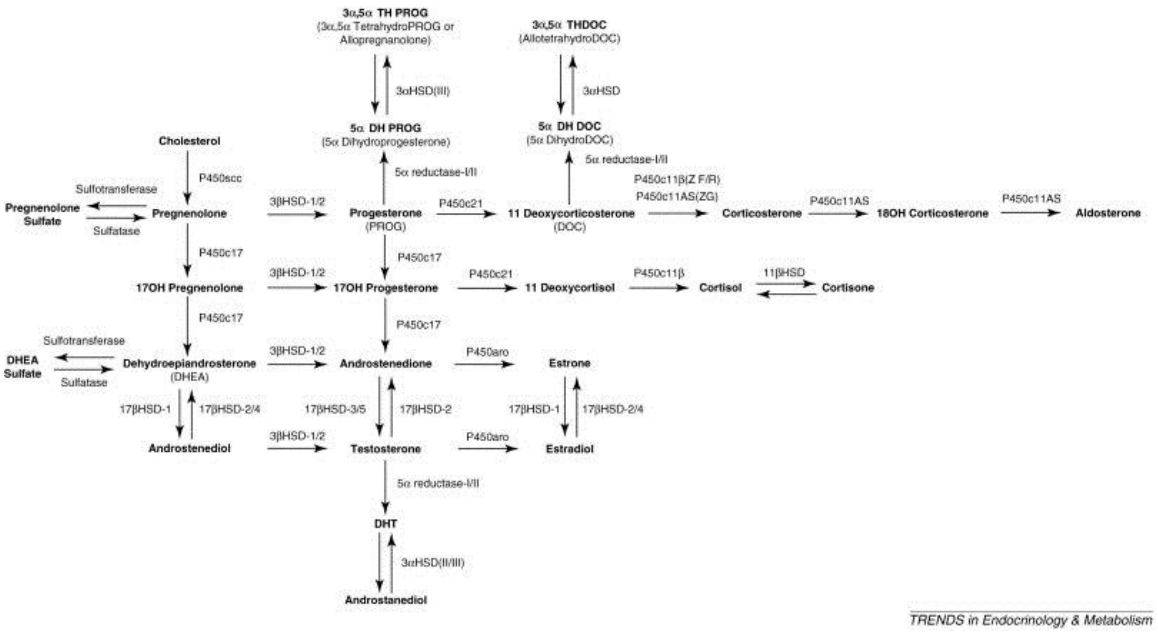

Figure 1.1. Progesterone Metabolism

Pregnenolone, a first-order metabolite of progesterone, has been shown to potentiate the activation of GABAa receptor evoked membrane currents up to seven times the baseline level (Callachan et al., 1987). At high enough levels, pregnenolone can directly activate the GABAa receptor (Callachan et al., 1987). This effect has been replicated (Smith et al., 1987) and also extended to show that physiologic levels of progesterone (conceivably after conversion to resulting metabolites) significantly suppress glutamate excitation (Smith et al., 1987). These in-vitro studies were later confirmed by in-vivo rodent models (Smith et al., 1989; Melchior & Ritzmann, 1994; Frye et al., 2004). Smith et al. (1989) measured Purkinje cell firing rates in the rat cerebellum and showed that, after 9-12 minute latency, firing rates compared to control were decreased in locomotion as a result of progesterone administration. In similar studies, both Melchior & Ritzman (1994) and Frye et al. (2004) demonstrated that pregnenolone enhanced motor activity in various maze and open field-type challenges

compared to control or placebo mice. The summation of previous literature indicates that progesterone metabolites have inhibitory effects on the nervous system, though the effect on the organism may be an increase of gross motor activity.

Estradiol has been shown to enhance dopamine release in the striatum of female rats (Becker, 1990; Xiao et al., 2003), which should result in an increase in ascending motor input to the motor cortex. In the absence of estradiol, GABAa binding is increased in the substantia nigra, pars reticulata, striatum, nucleus accumbens and entopeduncular nucleus, all of which are restored to normal levels with estradiol administration (Bossé & DiPaolo, 1996). The net effect of increased motor activity is a result of estradiol binding to ERα sites on GABAergic neurons, inhibiting GABA release (Schultz et al., 2009). Smith et al. (1989) confirm this hypothesis by showing that estradiol augments cerebellar neuron discharge during rat locomotion.

Effect of Sex Hormones/Menstrual Cycle on the Motor System

To date, there are is no published data indicating sex hormones or the menstrual cycle affect voluntary movement generation. However, a number of studies have examined the effect of the menstrual cycle and ovarian hormones on cortical excitability utilizing transcranial magnetic stimulation (TMS). Smith et al. (1999) first reported that normally menstruating females exhibited an increase in motor cortical inhibition at the luteal (mid-luteal) phase of the menstrual cycle compared to the follicular phase. This study was later followed up with a study which examined motor cortical excitation at the early follicular, late follicular and mid-luteal phases in order to examine more closely the interaction between progesterone and estradiol on the corticospinal tract. The latter study demonstrated that it was the late follicular phase which exhibited lower levels of inhibition compared to early follicular and mid-luteal phases (Smith et al., 2002). A

separate case study has also indicated that administration of exogenous progesterone increases cortical inhibition (Herzog et al., 2001). Based on the available literature, it can be surmised that there is an effect of sex hormones upon the corticospinal tract; however, the exact mechanism and which hormones cause these effects is still being elucidated.

Assessment of the Neuronal Connection between Motor Units

A signal coherence analysis examines the rhythmic frequency behavior of motor units. Coherence between two bioelectric signals indicates that they have a common oscillating neuronal origin (Farmer et al., 1993; Baker et al., 1997). Differential results in different frequency bands at menstrual phases demonstrate that neuronal interconnections are modulated by changes in sex hormones in humans.

The frequency domain analysis is well suited to systems which exhibit systemic rhythmic behavior and can be run on time-series as well as point-process data. Coherence provides a bounded (range 0-1) measure of the linear association between two processes (Amjad et al., 1997). The underlying theory is that coherent frequency oscillations in the muscle are indicative of neuron oscillations centrally, either in the motor cortex or lower cortical areas. Animal studies have verified this assumption by directly recording oscillatory bursts from the monkey motor cortex and EMG from the forearm during a reaching task and found coherence in the 20-30 Hz range (Baker et al., 1997). The animal studies were then extended to humans using surface EMG and magnetoencephalography, demonstrating coherence in the 15-30 Hz during a holding task (Kilner et al., 1999). Having firmly established the signal coherence link between cortical and muscular oscillations, the field has thusly concluded that if two motor units exhibit a coherent relationship they have a common neuronal oscillator (Farmer et al., 1993). Motor units in the extensor carpi radialis have coherence in the 6-12 Hz band

during extension movements, this coherence is weaker during a steady maintenance of force (Kakuda et al., 1999). Cross-sectional studies have indicated that the first dorsal interosseous muscle coherence is higher subjects unskilled in fine motor control of the muscle compared to highly skilled musician subjects (Semmler et al., 2004). These findings suggest that cortical inhibition to maintain fine motor control alters coherence of motor units within a muscle.

In the field of motor control, coherence is commonly assessed in discrete bands. The common drive band, 0-5 Hz, is indicative of common spinal origin and a common discharge rate modulation (De Luca & Erim, 1994; Myers et al., 2004; Lowery et al., 2007). A second band is attributed to physiological tremor, 5-12 Hz (Conway et al., 1995; Amjad et al., 1997). The beta band, 15-35 Hz, is indicative of a common origin at the cortical level (Conway et al., 1995; Baker et al., 1997). The Piper band, 35-60 Hz, appears to be a cortical circuit related to force generation greater than 60% of maximum (Brown et al., 1998) and preliminary evidence suggests the gamma band, 60-90 Hz, is related to a common brainstem origin (Spauschus et al., 1999).

Moreover, the entrainment, or coherence, of EEG signals from various scalp locations is indicative of common axonal connections, the length of which can be estimated based upon the cumulant density lag of the signal (Thatcher et al., 1986). While signal coherence has been used extensively in the motor control and EEG fields, the technique has never been applied to synergistic but separate nervous systems to assess their entrainment for common CNS origin.

Parallel Activity of the Motor and Cardiovascular Systems

Cardiovascular changes, determined by changes in ECG R-R interval, occur within 300 ms of exercise onset (Williamson et al., 1995). Furthermore, sympathetic

nerve activity to the skin increases prior to or in concert with the onset of force generation during both actual force generation and simulated force generation (Leuenberger et al., 2003). These findings suggest that the motor and autonomic nervous systems can be activated in parallel and have a probable common neuronal origin.

With the resurgence of surgical techniques treating Parkinson's and other movement diseases with intracerebral electrical stimulation, researchers have gained a new avenue to explore the interaction between movement generation and cardiovascular changes in awake human models. Direct, high frequency simulation (>90 Hz) of the thalamus, subthalamic nucleus and substantia nigra all increase heart rate, mean atrial pressure and facilitate movement in awake patients, demonstrating that deep brain stimulation can activate locomotion and the cardiovascular system in parallel (Thornton et al., 2002). When directly recording from the periaqueductal grey via indwelling electrodes, increased signal power in the 12-25 Hz and 60-90 Hz bands was observed during exercise anticipation (43% increase and 32% increase, respectively) and during exercise (87% increase and 109% increase, respectively) (Green et al., 2007). Given the parallel activation of the two systems and their reliance upon neurotransmitter systems modulated by sex hormones, it is likely that the menstrual cycle causes changes that are reflected in the motor and autonomic systems.

Heart Rate Variability

Heart rate variability (HRV) has been explored extensively since it was shown that the variability in canine heart rate can be extensively modulated by blockades in sympathetic, parasympathetic and renin-angiotensin systems (Akselrod et al., 1981). Since this time, the variability of heart rate (or heart period) rhythms has been associated with numerous factors and used as an indicator for various morbidities and clinical

outcomes. HRV has seen such extensive use in research and clinical settings that, in 1996, the Task Force of the European Society of Cardiology and North American Society of Pacing and Electrophysiology released a joint position statement regarding the standardized use and representation of HRV in research and clinical settings (Electrophysiology, 1996). HRV is calculated in the time domain, typically in standard deviation or coefficient of variation, or in the frequency domain, using the fast-Fourier transform. The derived power density spectrum is then broken down into frequency bands of total power (TP, 0.00066-0.40 Hz), very-low-frequency (VLF, 0.0033-0.04 Hz), low-frequency (LF, 0.04-0.15 Hz), and high-frequency power spectra (HF, 0.15-0.40 Hz).

The seminal work published by Akselrod (1981) demonstrated that the HF portion of the HRV spectrum was abolished with a parasympathetic blockade and the LF power spectrum was abolished by a combination of sympathetic and parasympathetic blockades. A study in humans determined, when moving from a supine to standing position, that the increase in LF was strongly mediated by sympathetic outflow, a suspected baroreflex response (Pomeranz et al., 1985). A principle component analysis of HRV and vagal tone at rest determined HRV standard deviation, HRV coefficient of variation, HRV mean, HF power and HF normalized power all contained solely the principle component of vagal tone; LF power and LF normalized power were found to contain a second component unrelated to vagal tone (Hayano et al., 1991). Multiple techniques, including the "gold standard" Oxford technique, have indicated that LF is predominately modulated by the baroreceptor-cardiovagal gain (Rahman et al., 2011). While it is clear that spectral parameters of HRV are strongly indicative of vagal control of the autonomic nervous system, there has been disagreement within the field whether or not it can be used as a non-invasive indicator of sympathetic activity.

Summary

It is apparent that sex hormones which normally oscillate across the menstrual cycle can have profound effects on neurotransmitter systems. A number of non-invasive or minimally invasive procedures and techniques can be employed to assess the peripheral effects of menstrual cycle changes on the nervous system. Studies utilizing the aforementioned techniques will provide evidence of sex hormone effects in humans and provide context to previous literature which has previously pooled the genders or not accounted for menstrual cycle phase.

[1]Chapter 2: Menstrual Cycle Mediates Vastus Medialis and Vastus Medialis Oblique Muscle Activity

ABSTRACT

Sports medicine professionals commonly describe two functionally different units of the vastus medialis (VM), the VM and the vastus medialis oblique (VMO), but the anatomical support is equivocal. The functional difference of the VMO is principle to rehabilitation programs designed to alleviate anterior knee pain, a pathology which is known to have a greater occurrence in women. The purpose of this study was to determine if the motor units of the VM and VMO are differentially recruited and if this recruitment pattern has an effect of sex or menstrual cycle phase. Single motor unit recordings from the VM and VMO were obtained for men and women during an isometric ramp knee extension. Eleven men were tested once. Seven women were tested during 5 different phases of the menstrual cycle, determined by basal body temperature mapping. The recruitment threshold and initial firing rate at recruitment were determined from 510 motor unit recordings. Initial firing rate was lower in the VMO than in the VM in women ($P < 0.001$), but not men. There was no difference in recruitment thresholds for the VM and VMO in either sex or across the menstrual cycle. There was a main effect of menstrual phase on initial firing rate, showing increases from the early follicular to late luteal phase ($P = 0.003$). The initial firing rate in the VMO was lower than in the

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Y-L Peng contributed to data collection, analysis, data interpretation and critically revising of the manuscript.
AC Hackney contributed to experimental conception and design and critically revising of the manuscript.
L Griffin contributed to experimental conception and design and critically revising of the manuscript.

VM during ovulatory (P = 0.009) and mid luteal (P = 0.009) phases. Thus, the relative control of the VM and VMO changes across the menstrual cycle. This could influence patellar pathologies which have a higher incidence in women.

INTRODUCTION

Clinical sports medicine textbooks commonly refer to two anatomically distinct portions of the vastus medialis (VM); the proximal portion is the VM and the distal 10% is termed the vastus medialis oblique (VMO)(Peeler et al., 2005). This distinction is made on the grounds that the VMO pennation runs oblique to the patella and thus has different functional properties than the proximal VM which has fibers running more longitudinal to the patella (Farahmand et al., 1998; Peeler et al., 2005). Anatomical studies have also shown that while the middle VM and VMO are both innervated by nerve roots originating from L1, L2 and L3, the VMO is innervated by a greater number of terminal nerve branches than the VM (Thiranagama, 1990). Recent cadaveric studies have indicated that after accounting for limb length discrepancies between subjects, the differences in pennation between the VM and VMO were reduced and the previously reported fibrofascial plane dividing the muscles is an anatomical rarity (Peeler et al., 2005). There is presently insufficient quality evidence to be certain that the VM and VMO are anatomically and functionally different muscles and no study has shown that the two muscles can be differentially recruited for their theorized different actions.

The VM is estimated to generate 25% of the knee extension force (Farahmand et al., 1998) while the VMO medially vectors the patella (Prentice, 2004; Magee, 2008) because of its insertion on the medial border (Peeler et al., 2005). The basic, however unsubstantiated, functional differences between the VM and VMO are the crux of rehabilitation protocols for patellofemoral pain syndrome and chondromalacia patella

(Laprade et al., 1998). If the VM and VMO are functionally different muscles, it is vital to understand how their differential activation may contribute to patellar stability because knee pain is the most commonly reported joint pain the United States of America (CDC, 2008).

There is a higher incidence of patellofemoral pain in women (Boling et al., 2010). If the VM and VMO are controlled independently, the large sex hormone oscillations in women may cause a differential activation of VM and VMO motor units (MUs) because progesterone and estradiol are known to affect neurotransmitter function (Callachan et al., 1987; Smith et al., 1987; Smith et al., 1989; Woolley et al., 1997). The enigmatic pathophysiology of patellofemoral pain syndrome and insufficient cadaveric evidence demonstrating functional differences in the VM and VMO have left a need for electromyography studies to examine VM and VMO activation patterns (Smith et al., 2009) and their differential effects of sex.

Only the recording of single MUs can conclusively demonstrate differences in descending drive and changes in MU recruitment because the surface electromyogram is subject to superimposition of MU action potentials, individual action potential shape and signal cross-talk. The goals of this study were two-fold: 1) to determine if the VM and VMO MUs can be differentially recruited and 2) to determine if motor unit firing patterns of recruitment are altered as an effect of sex or the menstrual cycle.

METHODS

Participants and Ethical Approval

Eleven young men (24.6±5.1 years) and seven young eumenorrheic women (24.9±4.3 years) participated in the study. Men participated in one study visit, all of which were conducted at 10:00 am. The women participated in five study visits at

defined points in the menstrual cycle: early follicular, late follicular, ovulation, mid luteal and late luteal. All women had their data collected in the morning. The time of data collection was standardized within each participant. Inclusion criteria for all participants were the absence of neurologic, cardiovascular, endocrine or metabolic disorders, previous leg surgery, immobilizations, arthritis, or chronic injury to the dominant leg. Additionally, the female participants must have been hormonal contraception naïve for at least 6 months prior to testing and have a history of clinically normal menstrual cycles. All participants gave their informed consent in accordance with the Helsinki Declaration and all experimental procedures were approved by the University of Texas at Austin Institutional Review Board.

Determination of Menstrual Cycle Phase

Menstrual cycle phase was determined by the basal body temperature mapping technique (de Mouzon et al., 1984). The cycle was mapped for one cycle prior to testing, continually monitored during the testing period and then verified upon completion of the study. Participants were instructed to take their temperature with an oral thermometer (BD Basal, Franklin Lakes, NJ) every morning before arising and consuming any beverage. The ovulatory phase was defined as the first day with a sustained temperature rise after basal body temperature nadir (de Mouzon et al., 1984) (see Figure 2.1). If an initial temperature map was not clearly defined, the participant performed a second cycle map prior to admission to the testing portion of the study. If the second map was not well defined, the participant did not enter data collection. The body temperature map was first assessed by a trained investigator (MST) and then confirmed by a senior investigator (ACH) with 25 years of research experience and 17 peer-reviewed publications utilizing the technique (A.C. Hackney, personal communication). While the low precision of the

basal body temperature technique in predicting exact ovulation day has been noted (Ecochard et al., 2001), it is effective in determining the length of the follicular and luteal phases as well as total cycle length and is used extensively in many clinical settings (Barron & Fehring, 2005). An example of the basal body temperature map from one subject is shown in Figure 2.1.

Data during each phase were collected in a pseudo-counterbalanced design with two women starting data collection in early follicular, two in late luteal and one in each phase of early late follicular, ovulation and mid luteal. The mid luteal phase for one participant was removed from analysis because they exhibited a short luteal defect and thus progesterone levels were inappropriately low for that phase (Daya et al., 1988); however, the data from that subject's other trials was appropriate because the late luteal trial was collected in the preceding cycle. One participant was found to be anovulatory in their last trial of data collection (ovulatory phase); therefore, that participant only had four trials collected because the data collection started at the mid luteal phase. Anovulatory status was defined as a lack of biphasic response in basal body temperature and abnormal cycle length.

Experimental Protocol

All data collection was performed in the climate controlled Neuromuscular Physiology Laboratory at the University of Texas at Austin. Participants were instructed to not perform strenuous physical activity or ingest food containing large amounts of phytoestrogens 48 hours prior to testing. Additionally, the participants were instructed to avoid alcohol and caffeine for eight hours prior to the visit and any food or beverage, except water, two hours prior to their study visit.

Participants were seated in an adjustable chair with the dominant hip and knee fixed at 90º. Leg dominance was determined by asking the participant which leg they would typically kick a soccer ball (left: 1, right: 6). The waist and dominant thigh were immobilized with pads and straps. Next, the individual performed a light warm up consisting of 12 dynamic submaximal knee extensions without resistance. The dominant ankle was then strapped to a padded restraint attached to the strain gauge (Entran Sensors & Electronics, Fairfield, NJ). The participant was instructed to perform three isometric maximal voluntary contractions (MVC) of the knee extensor muscles for 3 seconds. All MVCs were separated by at least 60 seconds of rest. The average of the three MVCs for that trial was used for calculations in the ramp protocol.

After completion of the MVCs, one bipolar intramuscular insulated stainless steel fine-wire electrode (0.002 mm diameter recording area, California Fine Wire Company, Grover Beach, CA) was inserted in the VMO and a second was inserted into the VM. Electrode placement was performed with a 25 gauge needle, 16 mm in length. The tip of the electrode was placed approximately 5-10 mm below the skin. For the purposes of electrode placement, VMO electrode was inserted immediately medial to the patella and the VM was defined as the area 7 cm superior to the VMO insertion point. The signals from the fine-wire electrodes were pre-amplified and bandpass filtered at 8 Hz – 3.12 kHz with a gain of 330 (B&L Engineering, Tustin, CA). An adhesive pre-gelled Ag/AgCl surface electrode of 5 mm diameter was placed over the ipsilateral patella and used as a ground. The participant then practiced performing a stable ramp contraction up to 30% of MVC. The participant was situated with a computer screen facing them with only their target force and force generation provided as visual feedback. They were instructed to trace a line on the screen with a rate of rise of 7.5% MVC per second up to 30% MVC and then hold the 30% MVC force for 3 seconds. The 7.5% rate of rise and

ramp contraction to 30% was chosen based on a pilot study which demonstrated these to have clearly defined MUs, low levels of discharge variability and limited MU superimposition. The participant practiced the ramp task 3-6 times each study visit before the trial used for data collection to ensure smooth force generation. The data collection trial was separated from the practice ramp contractions by a minimum of 60 seconds. Data for electromyography (EMG) and force was A/D converted (Micro 1401 Cambridge Electronic Design, Cambridge, England) and collected through Spike2 (version 5.21, Cambridge Electronic Design, Cambridge, England). Force and intramuscular EMG were sampled at 1 kHz and 30 kHz, respectively.

Motor Unit Data Reduction

EMG data was analyzed off-line in Matlab (version 2010b, Mathworks, Natick, MA) and Spike2. The data was 100 Hz high-pass filtered using a 4th-order recursive Butterworth filter. MUs were visually assessed and identified based upon shape, amplitude and discharge timing. MU recruitment was defined by four consecutive discharges at regular intervals (Van Cutsem et al., 1998). No motor unit double discharges were detected. The relative force at which that first discharge was recorded was the recruitment threshold. The initial firing rate was the average of the first three interspike intervals converted into hertz.

Statistical Analysis

All statistical analysis was performed using SAS (version 9.2) with α set at 0.05. When necessary, adjustments for multiplicity were performed using the Bonferroni correction technique. All motor unit data was considered cross-sectional because different MUs were presumably sampled on each testing date. MU recruitment threshold and MU initial firing rate were both assessed in a two-step process. The first step pooled

all female MUs across the menstrual cycle phases for comparison against the male subjects. The pooled data was used to determine if there was a difference in recruitment threshold and initial firing rate of the VM and VMO muscles between males and eumenorrheic women. The second step eliminated the male data and analyzed only the recruitment threshold and initial firing rate of the VM and VMO across the menstrual cycle.

Since recruitment threshold is a non-normal bounded measure (0-30) and exhibits a right-skewed distribution, a Mann-Whitney U test was used to test the distribution differences between the VM and VMO for males and females and at each phase of the menstrual cycle.

The initial MU firing rate during the ramp contraction for the VM and VMO was assessed by 2x2 random subject-level effects ANCOVA to determine an effect of sex and a second 2x2 random-effects ANCOVA to determine an effect of menstrual phase. The force at which the MU was recruited was used as a covariate because previous literature suggests a correlation between the force output at the time of MU recruitment and the initial firing rate (Milner-Brown et al., 1973). This random-effects approach controls for possible intra-correlation within each subject and is a more conservative approach than utilizing a standard ANCOVA or ANOVA. After determination of omnibus significance, an interaction analysis was performed to assess the differences in VM and VMO initial firing rate at every level of sex or menstrual phase.

RESULTS

Motor Unit Recordings

A total of 510 MUs were recorded. The mean coefficient of variation for MU recordings was acceptable for initial firing rates (13.6±8.8%). 130 of the 510 MUs

(25.5%) were collected from male participants. For the MUs recorded from females across the menstrual cycle, the breakdown was: 76 (early follicular), 96 (late follicular), 67 (ovulation), 64 (mid luteal) and 77 (late luteal).

Motor Unit Recruitment Threshold

There was no difference in the recruitment threshold distribution of the VM and VMO for either males ($p=0.411$) or females ($p=0.338$) (Figure 2.2). There were also no differences in the recruitment threshold distributions observed between the VM and VMO at any of the menstrual phases (Table 2.1).

Motor Unit Firing Rate at Recruitment

The two-way ANCOVA for sex and muscle group determined a main effect for VM/VMO muscle group ($p=0.002$) but not sex ($p=0.834$) for initial MU firing rate. The subject-level random effects approach was validated with a small but significant random coefficient (0.369, $p=0.012$). An interaction analysis determined that this difference in VM and VMO firing rate was predominately driven by the female MUs ($p<0.001$) (Figure 2.3).

The two-way ANCOVA for menstrual phase and muscle group determined a main effect for VM/VMO muscle group ($p<0.001$) and menstrual phase ($p=0.003$). The subject-level random effect exhibited a significant random coefficient (0.579, $p=0.046$). The post-hoc interaction analysis determined that the difference between VM and VMO firing rate was significantly different in the ovulation ($p=0.009$) and mid luteal menstrual phases ($p=0.009$) (Figure 2.4).

DISCUSSION

This study demonstrates that there are differences in the rate-coding strategy of motor unit recruitment between the VM and VMO. The higher initial MU firing rate in

the VM versus the VMO was statistically significant in females, but not in males. This may be due to a smaller relative sample size in males, though greater differences in VM-to-VMO firing rates were observed in the females compared to the males (0.66 Hz vs. 0.47 Hz). To our knowledge, this is the first study to show that the VM and VMO are differentially activated during simple knee extension and are thus muscles which receive different neurological drive. Furthermore, the hormones which oscillate across the menstrual cycle appear to promote the differential activation of the two muscles. The menstrual cycle timing of the VM/VMO differentiation, in the ovulatory and mid luteal phases, suggests that progesterone is the strongest mediator of this differentiation with a possible secondary action of estradiol.

The findings of this study in combination with the different neurological innervation (Thiranagama, 1990) and difference in pennation angles (Farahmand et al., 1998) support a functional difference between the VM and VMO despite a lack of a distinct fibrofascial division between the muscles (Peeler et al., 2005). Specifically, the present study answers a recent call from the anatomical community for electromyography studies to determine if in vivo functional differences exist between the VM and VMO (Smith et al., 2009). Our study also supports sports medicine and orthopedic textbooks which differentiate the muscles into different functional units and leaves open the possibility that the two muscles may have different biomechanical purposes (Prentice, 2004; Magee, 2008).

The ~1 Hz difference in MU firing rates observed in the ovulatory and luteal phases may seem small when viewed in isolation, but this change can substantially impact muscular force generation. In the soleus, a 1 Hz decrease in MU discharge at recruitment can cause a 10% decrease in force generation (Oya et al., 2009). The changes in MU discharge in the current study are greater than the decrease in MU firing

observed after 6-8 weeks of immobilization (Duchateau & Hainaut, 1990). The ~1 Hz change is also commensurate with the change in MU discharge from the VMO and vastus lateralis (VL) when knee pain is induced (Tucker et al., 2009). Therefore, we can surmise that our data indicate a possible change in the relative force output of these muscles as a result alterations in MU discharge across the menstrual cycle.

Oscillations of estradiol and progesterone at the plasma level affect the central nervous system because steroids easily traverse the blood-brain barrier due to their high lipid solubility (Stoffel-Wagner, 2001). Estradiol binds to estrogen receptor α sites on γ-aminobutyric acid (GABA) releasing neurons resulting in decreased GABA transmission (Schultz et al., 2009) and enhanced cerebellar neuron discharge during rodent locomotion (Smith et al., 1989). In addition to decreasing GABA release, estradiol acts to sensitize NMDA receptors to glutamate in pyramidal cells (Woolley et al., 1997). While estradiol is excitatory in nature, progesterone metabolites can directly activate GABAA receptors (Callachan et al., 1987; Smith et al., 1987). However, progesterone has also been shown to decrease cerebellum Purkinje cell discharge rates in rats during locomotion (Smith et al., 1989). Since Purkinje cells inhibit the cerebellar nuclei, progesterone could also have a net excitatory effect on motor output. The first-order metabolite of progesterone, pregnenolone, enhances motor activity in various maze and open field-type challenges compared to control mice (Frye et al., 2004). These hormones could also affect the basal ganglia–ventral anterior/ventrolateral thalamic pathway which is known to initiate and modulate voluntarily generated and visually guided movements (MacMillan et al., 2004), similar to the visually guided ramp contraction performed in the present study. This pathway is a hybrid neurotransmitter pathway (Percheron et al., 1996), affected by changes in glutaminergic and GABAergic activity, both of which are modified by progesterone and estradiol (Callachan et al., 1987; Smith et al., 1987; Woolley et al.,

1997; Schultz et al., 2009). This hybrid pathway may offer an avenue for sex hormones to differentially affect MU discharge patterns across the menstrual cycle.

The current study is the first to demonstrate that voluntary MU activation in any muscle can be modulated by the menstrual cycle. Transcranial magnetic stimulation (TMS) can stimulate the orderly recruitment of motor units via the corticospinal tract (Bawa & Lemon, 1993), but it does not account for changes in subcortical or cerebellar brain areas. TMS studies have shown an initial depression in corticospinal excitability in the early follicular phase (Smith et al., 2002). This is also the time when MU firing rates were observed to be the lowest in the present study. There is also lower corticospinal tract excitability at the mid luteal phase (Smith et al., 2002). We did not find this to be the case for the VM firing rates. It is apparent that menstrual cycle sex hormones profoundly affect excitability of the central nervous system. Using H-reflexes as a metric for excitability at the spinal level, Hoffman et al. (2008) demonstrated that there was no change across the menstrual cycle. This indicates that changes in central nervous system excitability likely occur at the cortical and/or subcortical levels. These changes may manifest themselves to a different degree in the VM and the VMO muscles that extend the knee and stabilize the patella.

It should be noted that muscle force is produced by both motor unit firing rate and the total number of motor units recruited. We did not measure the total number of motor units recruited in each muscle, thus we are unable to estimate the relative forces produced by these muscles. If the primary function of the VMO is to stabilize the patella and the primary function of the VM is to assist with leg extension, presumably more force would be required by the VM than the VMO. It is possible that the overall firing rates were higher in the VM because higher forces were required to produce leg extension than patellar stabilization. The differences in firing rate between the two muscles could also be

indicative of differences in fiber type. The VM tends to have higher proportions of Type 1 and Type 2a muscle fibers compared to the VMO, though there is substantial inter-subject variability (Travnik et al., 1995) and no differences in MU recruitment thresholds were observed in the present study. Nevertheless, if the VMO is composed of a greater proportion of the larger Type 2 muscle fibers, lower firing rates would be required to reach twitch fusion. It is also possible that there are differences in MU rate-coding and recruitment strategies between the two muscles.

We did not find statistically significant differences in MU firing rates between the sexes. While we are not aware of research demonstrating significant differences in VM/VMO muscle fiber types between the sexes, previous studies in other muscles have indicated that females may have a lower proportion of Type 2 fibers compared to males (Miller et al., 1993). Previous work in the animal model has indicated that MUs recorded in males fire more rapidly than those in females (English & Widmer, 2003), a finding our study fails to confirm. Miller et al. (1993) also found no difference between the sexes when examining total number of MUs, MU size or MU activity in the VM. Some differentiation between the sexes has been noted in the architecture of the soleus muscle (Chow et al., 2000) and while we are unable to discount this possibility within the VM and VMO of our sample population, there is no reason to expect that any change in muscle architecture occurred across the menstrual cycle.

Moreover, the effects of hormonal contraception on muscle activation and recruitment patterns are unknown and in need of future research. Hormonal contraception decreases circulating estradiol and progesterone (Coney & DelConte, 1999); however, it is unknown if the synthetic sex hormone analogues have physiological actions on the neurotransmitter system similar to their endogenous counterparts.

Furthermore, the effects of amenorrhea, oligomenorrhea, pregnancy and menopause on MU activation and recruitment patterns are also potential areas of future research.

In summary, our study shows that MU activation differs between the VM and VMO and across the menstrual cycle. The difference in activation is highest during the ovulatory and mid luteal phases and the general activation of both the VM and VMO are increased in the late luteal phase of the menstrual cycle.

	Vastus Medialis (% MVC)	Vastus Medialis Oblique (% MVC)	P-Value
Early Follicular Phase	8.7	8.7	0.72
Late Follicular Phase	10.9	8.6	0.38
Ovulatory Phase	12.4	8.5	0.26
Mid Luteal Phase	10.7	10.7	0.84
Late Luteal Phase	10.1	7.1	0.58

Table 2.1. Median Recruitment Thresholds During the Menstrual Cycle

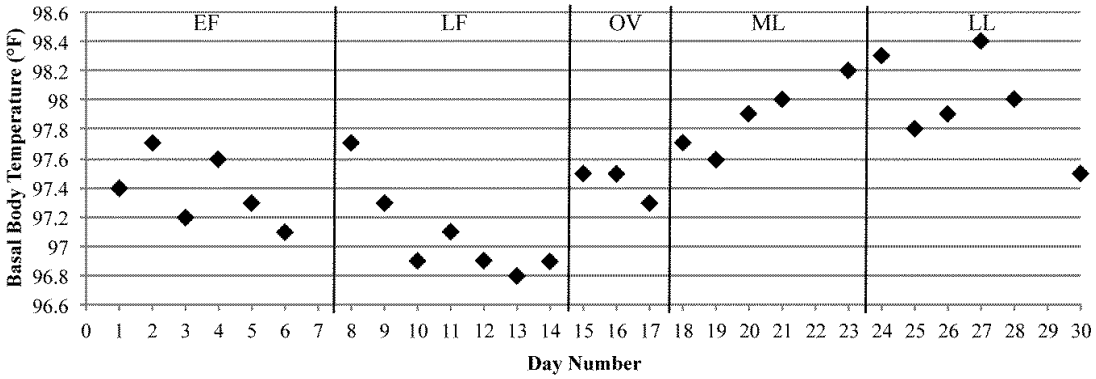


Figure 2.1. Example of a Basal Body Temperature Map

Phases are indicated (Early follicular=EF; Late Follicular=LF; Ovulatory=OV; Mid Luteal=ML; Late Luteal=LL).

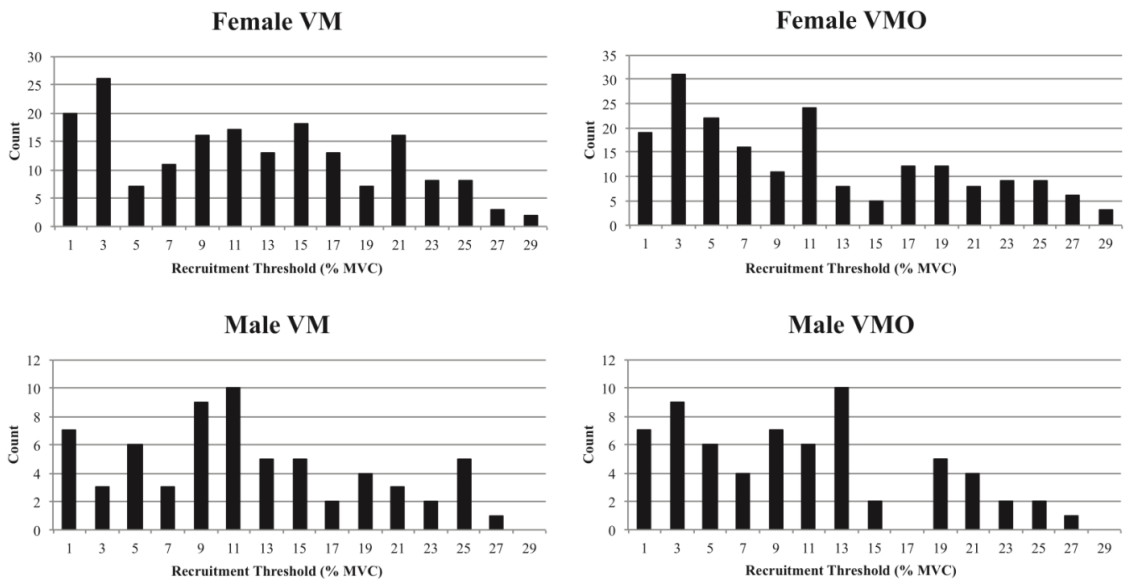


Figure 2.2. Recruitment Threshold Distributions by Sex

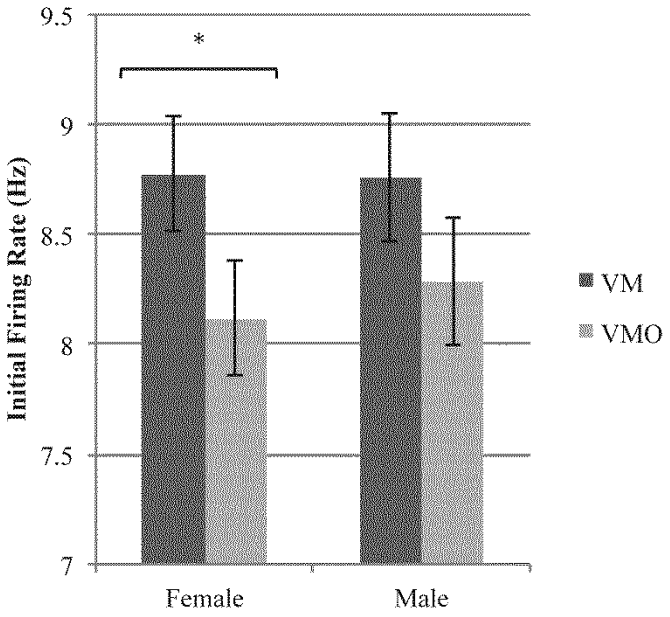


Figure 2.3. Motor Unit Firing Rates at Recruitment by Sex

VM firing rate is significantly higher than VMO for female subjects (* indicates $p < 0.05$).

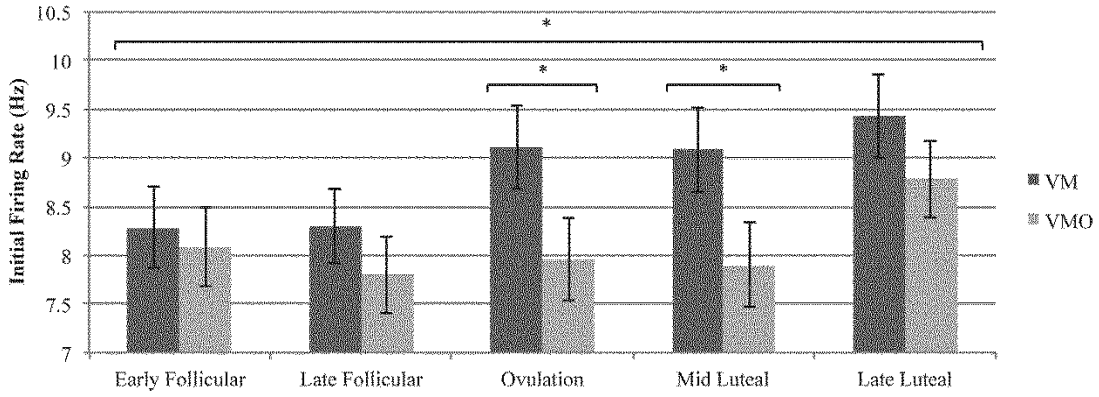


Figure 2.4. Motor Unit Firing Rates at Recruitment by Menstrual Phase

The VM firing rate is significantly higher than VMO firing rate at the ovulatory and late luteal phases. Main effects for increases in firing rate were also observed across the menstrual cycle (* indicates $p < 0.05$).

Chapter 3: Entrainment of Motor Activity in the Vastus Medialis and Vastus Medialis Oblique: Effect of Sex and the Menstrual Cycle

ABSTRACT

The existence the vastus medialis oblique (VMO) as a distal functional unit of the vastus medialis (VM) is disputed among anatomists and physical medicine clinicians. Delayed VMO onset timing is an effective predictor of future development of patellofemoral pain syndrome, known to have higher rates in females. The goal of this study was to examine oscillatory patterns of VM and VMO motor activity in males and females as well as across the menstrual cycle to ascertain the control properties of the motor units (MUs) within muscle sub-sections. Single MUs and surface electromyogram (EMG) were collected from the VMO and VM of 9 males and 9 females. The males were tested once and the females were tested during 5 menstrual cycle phases. A coherence analysis was run on the MU-MU and MU-EMG data and multilevel linear and logistic models were used to assess statistical significance. Males had greater MU-MU spectral power in the common drive band (0-5 Hz) and greater MU-EMG spectral power in the tremor band (5-12 Hz) compared to females. Males had a 741% (MU-MU pair) and 256% (MU-EMG) greater odds of having coherent oscillations in the common drive band than females. There is little evidence that the menstrual cycle affects coherent oscillations. Compared to MUs in the VM, VMO and VM/VMO MU pairs have 228% and 212% greater odds of having coherent oscillations in the beta band (15-35 Hz). The VM and VMO are neurologically different muscles.

INTRODUCTION

The existence and functional difference of the vastus medialis oblique (VMO) as a component of the vastus medialis (VM) muscle is controversial among both anatomists (Peeler et al., 2005; Smith et al., 2009; Engelina et al., 2014) and physical medicine

clinicians (Mirzabeigi et al., 1999; Boling et al., 2006a; Boling et al., 2006b; Bennell et al., 2010). There is conclusive evidence, from both in vivo and cadaver studies, that the VMO has a greater angle of pennation than the VM (Smith et al., 2009; Engelina et al., 2014). A cadaver study using primarily older bodies (79±12 years of age) indicated that a low proportion of VMO fibers attach to the medial border of the patella (Peeler et al., 2005); however, an in vivo study of younger subjects (Range: 20-30 years of age) using ultrasound reported an average of 57.8% of the medial patella was inserted upon by the VMO (Engelina et al., 2014). In a meta-analysis, 41% of cadavers exhibited two distinct nerve trunks, one feeding the proximal VM and one feeding the distal VM/VMO (Smith et al., 2009). The VMO has also been reported to be supplied by a greater number of terminal nerve branches than the proximal VM (Thiranagama, 1990).

Clinicians have long known that VMO onset timing is delayed compared to other vasti muscles in patients suffering from patellofemoral pain syndrome (PFPS) (Voight & Wieder, 1991) and that effective rehabilitation decreases PFPS pain and "normalizes" VMO onset timing (Boling et al., 2006a). PFPS occurs in females more than twice as often as their male counterparts (Boling et al., 2010). Therefore, the root cause of increased PFPS rates in women may be due to differences in the modulation of muscles which control patellar tracking. We have previously shown that, at recruitment, the motor unit (MU) discharge patterns of the VM and VMO are different between the sexes and that changes in the MU discharge rate occur across the menstrual cycle (Tenan et al., 2013). The large-scale fluctuations in estradiol and progesterone occurring across the menstrual cycle are known to affect neurotransmitter activity in animal models (Callachan et al., 1987; Smith et al., 1987; Smith et al., 1989; Woolley et al., 1997). This may affect neuronal modulation of synergistic muscles controlling patellar tracking.

Electromyographic signal coherence between two muscles indicates that they have a common oscillating neuronal origin (Farmer et al., 1993; Baker et al., 1997). An investigation into the oscillatory coupling of the VM and VMO should definitively determine if a functional relationship exists between the two muscle areas at a neurological level. As there is a sex discrepancy in incidence of PFPS (Boling et al., 2010) and recruitment of the two muscles is different for the sexes and across the menstrual cycle (Tenan et al., 2013), the neurological relationship of the two muscles may also be different. Therefore, the goal of the present study was to examine the coherent oscillations of single MUs and surface electromyography (EMG) from the VM and VMO in both males and females as well as across the menstrual cycle.

METHODS

Participants and Ethical Approval

Nine males (24.8±5.3 years) and nine eumenorrheic women (24.7±4.5 years) participated in the study. Males participated in one study visit, all of which were conducted at 10:00 am. The females participated in five study visits during their menstrual cycle: early follicular, late follicular, ovulation, mid luteal and late luteal. All female data collection was performed in the morning and standardized within each participant. Inclusion criteria for all participants were the absence of neurologic, cardiovascular, endocrine or metabolic disorders, previous leg surgery, immobilizations, arthritis, or chronic injury to the dominant leg. Additionally, the female participants were hormonal contraception naïve for at least 6 months prior to testing and had a history of clinically normal menstrual cycles. All participants gave their informed consent and all experimental procedures were approved by the University of Texas at Austin Institutional Review Board.

Basal Body Temperature Determination of Menstrual Cycle Phase

Data was collected from the female study participants during the five phases of the menstrual cycle. The first point of data collection for each subject was randomized and resulted in a pseudo-counterbalanced design with participants starting data collection in the following distribution: 2 early follicular, 2 late follicular, 1 ovulatory, 2 mid luteal and 2 late luteal. Our method of determining menstrual cycle phase via basal body temperature (BBT) in this cohort has been described previously (Tenan et al., 2013; Tenan et al., 2014a).

Briefly, participants obtained their BBT via oral thermometer (BD Basal, Franklin Lakes, NJ) for one month prior to data collection. The biphasic response in BBT is characteristic of a normal menstrual cycle with ovulation being operationally defined as the nadir before the luteal phase temperature rise (de Mouzon et al., 1984). If the temperature map from the first month was not clearly defined, the participant performed a second cycle map before admission to the data collection portion of the study. If the second temperature map was not well-defined, the participant did not enter data collection. The BBT was first assessed and then subsequently confirmed independently by two trained investigators. One participant did not have their EMG data analyzed during their last study visit in the mid luteal phase because they exhibited a short luteal defect; however, the data from that participant's other trials was included because the late luteal trial was collected in the preceding cycle. A second participant was anovulatory, defined by a lack of biphasic response in the BBT, in their last study visit during the ovulatory phase; therefore, that participant only had four study visits because their data collection started in the mid luteal phase. The total number of subject study visits in each phase and motor unit pairs collected can be reviewed in Table 3.1.

Experimental Protocol

All study visits were performed in the Neuromuscular Physiology Laboratory at the University of Texas at Austin. Participants were instructed to not perform strenuous physical activity or ingest food containing large amounts of phytoestrogens 48 hours prior to testing. Additionally, the participants were instructed to avoid alcohol and caffeine for eight hours prior to the visit and any food or beverage, except water, two hours prior to their study visit.

The experimental setup has been previously described (Tenan et al., 2013). Briefly, Participants were seated in an adjustable chair with the dominant hip and knee fixed at 90º. The waist and dominant thigh were immobilized with pads and straps. The participant performed 12 dynamic submaximal knee extensions without resistance before the dominant ankle was secured into a padded restraint attached to a strain gauge (Entran Sensors & Electronics, Fairfield, NJ). The participant performed three isometric maximal voluntary contractions (MVC) of the knee extensors, separated by 60 seconds of rest. The average of the three MVCs for that trial was used to ascertain the absolute force at which the participant would perform a sustained isometric knee extension during the test protocol.

After completing the MVCs, bipolar intramuscular insulated stainless steel fine-wire electrodes (0.002 mm diameter recording area, California Fine Wire Company, Grover Beach, CA) were inserted into the VM and VMO. Electrode placement was performed with a 25 gauge needle, 16 mm in length. The VMO insertion point was immediately medial to the patella and the VM was defined as the area 7 cm superior to the VMO insertion point.

Two adhesive pre-gelled Ag/AgCl surface electromyography (EMG) electrodes (5 mm diameter, 10 mm interelectrode distance) were placed 2-3 mm superior to VM fine-

wire insertion point in parallel with the theorized angle of muscle pennation. No surface EMG was collected from the VMO due to the small size of the muscle. A surface electrode ground was placed over the ipsilateral patella.

The participant then practiced a steady force ramp up to 30% MVC. Target force feedback was visually provided by a screen positioned directly in front of the participant at eye-level. The participant was asked to trace a line with their force output. For the data collection trial, the participant performed a force ramp up to 30% MVC and held that target force until they were instructed to terminate the exercise by the investigator. The exercise termination point was defined by either force oscillations greater than ±5% MVC or the inability to maintain force output for three seconds. Data for fine-wire EMG, surface EMG and force were A/D converted (Micro 1401 Cambridge Electronic Design, Cambridge, England) and collected through Spike2 (version 5.21, Cambridge Electronic Design, Cambridge, England). Force and surface EMG were sampled at 1 kHz and intramuscular EMG was sampled 30 kHz.

Motor Unit Data Reduction

All motor unit data reduction was performed in Spike2 (version 7.09a). Fine-wire EMG was band-pass filtered 100 Hz – 5 kHz with a 4th order Butterworth filter. Individual motor unit action potential trains were identified using Spike2's template matching algorithm. The classification was confirmed and edited by a singular investigator using Spike2's principal component analysis algorithm as well as a manual case analysis. When a motor unit discharged continuously for ≥20 seconds, it was exported at 1000 Hz for inclusion in the coherence analysis.

Coherence Analysis

Single motor units discharging concurrently for 20 seconds and surface EMG from the VM were assessed via a coherence analysis performed with Neurospec 2.0's core routines (Halliday et al., 1995) for Matlab (version R214a, MathWorks, Natick, Massachusetts). When a hybrid coherence analysis was performed using a MU spike train and surface EMG, the surface EMG was full-wave rectified and linearly detrended prior to analysis. The coherence analysis was performed on 20 seconds of MU and/or EMG data using a series of non-overlapping discrete Fourier transforms 1.024 seconds in length (Halliday et al., 1995). The resulting weighted periodogram estimate for the analysis had a frequency resolution of 0.98 Hz. For each coherence analysis, the power contained within five frequency bands was determined: common drive 0-5 Hz (De Luca & Erim, 1994; Myers et al., 2004; Lowery et al., 2007), physiological force tremor 5-12 Hz (Conway et al., 1995; Amjad et al., 1997), beta band 15-35 Hz (Conway et al., 1995; Baker et al., 1997), Piper band 35-60 Hz (Brown et al., 1998) and gamma band 60-90 Hz (Spauschus et al., 1999). Using the 95% confidence limit calculated by Neurospec 2.0, it was determined if there were oscillations within each band that were unlikely to be due to random noise or chance (ie. oscillations above the 95% confidence limit).

Statistical Analysis

Two statistical approaches were taken to assess the neurological connection between VM and VMO MUs and the effect of sex or menstrual phase: 1) multilevel linear regression models were used to perform a population analysis of motor unit pairs while controlling for subject-level correlations of MU pairs and 2) multilevel logistic regression models were used to assess significant oscillations in a frequency band. The multilevel linear regression provides a weighted average of the strength of coherence within a frequency band; however, it may inappropriately "average" MU pairs which

have a common neural origin with MU pairs that do not have a common origin. The multilevel logistic regression does not provide information regarding the absolute strength of MU coherence, but it indicates the likelihood there will be MU pairs that have common oscillations in a frequency band and thus have a common neuronal origin.

When a hybrid MU and surface EMG coherence analysis was performed, similar statistical approaches were utilized. The multilevel structure controlling for subject-level correlations was performed because previous research has indicated that the discharge patterns of single motor units are correlated within an individual (Tenan et al., 2014b). Early statistical models included an ANCOVA-style analysis to control for the time point at which the MU-MU and MU-EMG pair were recorded as early research on EMG-EMG coherence has indicated that fatigue may affect coherent activity (Boonstra et al., 2008). In the present study, the effect of time was never statistically significant and did not alter the interpretation of the results. Therefore, the time covariate was not included in the final models. The alpha-level for each regression was set at 0.05.

Multilevel Linear Regression

For each frequency band, the effects of sex, MU pair location (i.e. VM/VM, VMO/VMO or VM/VMO) and the interaction of these effects were assessed with a multilevel linear regression controlling for subject-level correlations using an unstructured covariance structure. When assessing the effects of sex, all female MU pairs were contrasted with those from male participants.

A similar approach was performed to assess the effect of the menstrual cycle. The male data was removed from the analysis and the menstrual cycle was assessed as the number of days prior or post ovulation, defined as BBT nadir.

When performing multilevel linear regression on the hybrid MU and surface EMG coherence data, similar analyses were performed to that of MU pair data. However, since all coherence analyses were MU/EMG, and there was no surface EMG from the VMO, the MU/EMG pair location variable contained one less category than the MU/MU analyses (i.e. MU-VM/EMG-VM and MU-VMO/EMG-VM).

The necessity of using a multilevel regression for MU coherence analyses was descriptively assessed with the intraclass correlation (Singer, 1998; Tenan et al., 2014b).

Multilevel Logistic Regression

For each frequency band, the odds of having a MU pair with significant oscillations was determined with sex and MU pair location as predictor variables. Initial modelling attempts also had an interaction effect of these terms, but the interaction caused quasi-complete separation of the variables leading to a termination of the maximum likelihood iteration process. The multilevel structure controlled for multiple MU pairs observed within each participant with an unstructured covariance structure.

The odds of MU pair significant oscillations across the menstrual cycle was assessed with days from ovulation, MU pair location and the interaction of these variables as predictors. When assessing the odds ratio results from the analysis, days from ovulation was observed in 7 day increments.

The multilevel logistic regression on hybrid MU and surface EMG was similar to the MU-MU analyses. For all logistic regressions, statistical significance was attained when the 95% confidence limits of the odds ratio did not include 1.

RESULTS

A total of 186 MU pairs and 159 MU-surface EMG pairs were used for the sex-based analyses and 164 MU pairs and 135 MU-surface EMG pairs were used for the menstrual cycle analyses. The distributions of these observations are in Table 3.1.

Multilevel Linear Regression Between Sexes

The intraclass correlation for MU pairs and MU-surface EMG pairs within individuals was notable in some analyses but not all (Table 3.2).

For MU-MU analyses, there was a significant main effect for sex in the common drive band ($p<0.01$), but no significant effect for MU pair location or interaction effect (Figure 3.1). Neither tremor, beta, Piper or gamma bands had any significant effects ($p>0.05$). However, MU pair location and the interaction of MU pair location and sex both trended towards significance in the gamma band ($p=0.07$, both). For MU-EMG analyses, there was a significant main effect for MU-EMG pair location (Figure 3.2) in the tremor band ($p<0.01$) and a trend towards significance for the interaction of location and sex in the same band ($p=0.08$). Additionally, there was a trend towards significance in common drive band for sex ($p=0.07$). There was no significant effects for other frequency bands and variables ($p>0.05$).

Multilevel Linear Regression across the Menstrual Cycle

When assessing the MU-MU coherence across the menstrual cycle, there was no apparent effect of either days from ovulation or MU pair location ($p>0.05$; Figure 3.3). The MU-EMG analysis in the tremor band indicated that VM coherence was significantly higher than coherence power from VM/VMO ($p=0.01$), the secondary interaction effect analysis showed that VM coherence was higher than VM/VMO one day prior to

ovulation and after ovulation ($p=0.05$; Figure 3.4). There was also a main effect for MU-EMG pair location in the beta band ($p<0.01$; Figure 3.4).

Multilevel Logistic Regression Between the Sexes

Males had significantly higher odds of having motor units with coherent oscillations in the common drive band in both the MU-MU and MU-EMG analyses (Figure 3.5, 95% CI not including 1). The MU-MU common drive odds ratio (OR) (8.41) indicated that males have 741% greater odds of having MU-MU pairs with coherent oscillations. The MU-EMG common drive odds ratio (3.56) indicated that males have 256% greater odds of having MU-EMG pairs with coherent oscillations.

VM/VMO MU-MU pairs are significantly different from VM MU-MU pairs in the tremor and beta bands (Figure 3.6) with odds ratios showing that there is 232% and 228% greater odds of having coherent oscillations in these bands (OR: 3.32 & 3.28). VMO MU-MU pairs also have 212% greater odds of having coherent oscillations in the beta band compared to VM MU-MU pairs (OR: 3.12). The MU-EMG analyses demonstrated that VM/VMO MU-EMG pairs in the tremor and beta bands have a 117% and 127% lower odds of having coherent oscillations compared to VM MU-EMG pairs (OR: 0.46 and 0.44, respectively).

Multilevel Logistic Regression across the Menstrual Cycle

In both the MU-MU and MU-EMG analyses, there was no significant effect of the menstrual cycle on muscle coherence (all 95% CI include 1; Figure 3.7). Holding menstrual cycle constant, the MU-MU analyses showed that VM/VMO pairs have 243% and 320% greater odds of having coherent oscillations in the tremor and beta band, respectively (OR: 3.43 and 4.20, respectively; Figure 3.8). Additionally, the VMO MU-MU pairs have 250% greater odds of having coherent oscillations in the beta band than

VM MU-MU pairs (OR: 3.50). The MU-EMG analyses indicate that, holding menstrual cycle constant, VM/VMO pairs have 156% lower odds of having coherent oscillations in the beta band compared to VM MU-EMG pairs (OR: 0.39; Figure 3.8).

DISCUSSION

There are systematic differences between neuronal oscillations of the sexes but not across the menstrual cycle. Males consistently have higher levels of coherent oscillations in the common drive band. Thus, motor unit pairs in the area of the VM and VMO have a higher level of common rate modulation (Myers et al., 2004; Lowery et al., 2007) and common neuronal origin at the spine (De Luca & Erim, 1994).

The second goal of the study was to determine if the VM and VMO are neurologically different muscles by examining the coherent oscillations of the MUs from the areas. The present study agrees with previous research from our group showing that the VM and VMO are differentially modulated (Tenan et al., 2013). This study further demonstrates that the differential modulation is contained primarily within the beta band with a secondary difference in the tremor band, indicating that the primary difference in neuronal origin is at the cortex (Conway et al., 1995; Baker et al., 1997).

Subject-Level Correlations

This study did not find consistent and robust subject-level correlations when examining coherent oscillatory patterns of MU pairs or MU-EMG pairs, contrary to previous research showing that the discharge rate of MUs was correlated within a subject (Kamen et al., 1995; Tenan et al., 2014b). The reason for this discrepancy is unclear. It is possible that while the descending motor drive and spinal mechanisms by which MUs discharge to create force are variable at the subject-level, the functional structure of the nervous system, as assessed by motor coherence, is not discernibly related to each

individual. It is clear that in many cases MU-MU and MU-EMG pairs can be considered statistically independent, though this assumption is sometimes violated (Table 3.2).

Sex and Menstrual Cycle

Both amplitude of motor coherence and the odds of having coherent oscillations in the common drive band were higher for males compared to females. This result is both statistically significant and practically significant indicating that males have 741% greater odds of having coherent oscillations compared to females. This finding explains our previous research which demonstrated that females, but not males, have different MU discharge rates for the VM and VMO at recruitment (Tenan et al., 2013). If males have a substantially higher level of common discharge rate modulation in these muscles, evidenced by higher common drive, then there is a neurological structural constraint to their differential modulation.

The common MU discharge rate modulation more likely observed in males may be a contributing factor to the lower rate of knee injuries in males (Arendt & Dick, 1995; Boling et al., 2010). If the VMO is activated in continuity with other vasti muscles during knee extension, this should provide stability to the patellofemoral joint. A lack of connectedness or discontinuity in VMO activation in relation to other vasti muscles may lead to more variable internal and external biomechanics, causing injury or pain.

Previous research has shown that the discharge patterns of the VM and VMO are altered across the menstrual cycle (Tenan et al., 2013). The present study does not demonstrate any systemic change in the oscillatory patterns of the VM and VMO occur across the menstrual cycle. There is some evidence that VM MU-EMG coherence in the tremor band is higher than VM/VMO pairs after ovulation, though this is not supported by the MU-MU coherence data. The fluctuation in initial discharge rate across the

menstrual cycle may be modulated by changes in corticospinal tract excitability without alterations to the physical connections within the tract.

Differential Vastus Medialis and Vastus Medialis Oblique Oscillations

The present study clearly demonstrates that the VM and VMO are neurologically different muscles. VMO and VM/VMO MU-MU pairs have 212% and 228% greater odds of having common oscillations in the beta band compared to VM pairs, indicating that MUs in the VMO are commonly modulated at the cortex (Baker et al., 1997). Additionally, the data suggest that the MUs with common neuronal origin in the distal portion of the vastus medialis complex occupy a larger geographic area than anticipated. This can be seen by the higher odds of having common oscillations in the beta and tremor bands for MUs sampled from both the VMO and VM locations compared to MUs sampled from the VM location alone. This hypothesis is supported by the MU-EMG coherence data. The surface EMG was collected just superior to the VM fine-wire insertion point; therefore, it is unsurprising that the MU-EMG VM pairs have higher odds of coherent oscillations than the more geographically separated VMO fine-wire insertion point. What is surprising is that the MU-EMG data reliably results in findings opposite from MU-MU data. This indicates that the more proximal motor activity is observed, the greater the functional connectivity and that the VM insertion point in the present study may be too distal to truly be considered not neurologically similar to the VMO. Indeed, VM/VMO MU-MU pairs commonly have higher odds of coherent oscillations than VM MU-MU pairs. Obviously, the neurologic architecture of the vastus medialis complex has not been fully elucidated, but needs to be considered in concert with the anatomical data to understand how this muscle complex functions in vivo.

Conclusions

In summary, males are more likely than females to have MUs that have a common rate modulation. There is little evidence that the menstrual cycle alters motor unit coherence within the vastus medialis complex. The motor units supplying the vastus medialis complex appear to be distributed according to their geographic location. Therefore, while sex affects the likelihood of functional connectivity at the spinal level, control of the sub-portions of the vastus medialis complex is distributed at the cortex.

	Males	Early Follicular	Late Follicular	Ovulation	Mid Luteal	Late Luteal
Participants	9	9	9	8	8	8
MU-MU Pairs	22	44	33	11	42	34
MU-EMG Pairs	24	33	29	19	29	25

Table 3.1. Number of Participants and MU-MU/MU-EMG Pairs

Frequency Band	Analysis	ICC
Common Drive	MU-MU	0.01
Common Drive	MU-EMG	0.01
Tremor Band	MU-MU	0.00
Tremor Band	MU-EMG	0.07
Beta Band	MU-MU	0.04
Beta Band	MU-EMG	0.17
Piper Band	MU-MU	0.00
Piper Band	MU-EMG	0.00
Gamma Band	MU-MU	0.00
Gamma Band	MU-EMG	0.06

Table 3.2. Subject-level Intraclass Correlations for Sex

Note: ICC = Intraclass Correlation.

Figure 3.1. MU-MU Coherence Power in Frequency Bands

Note: * indicates $p<0.05$

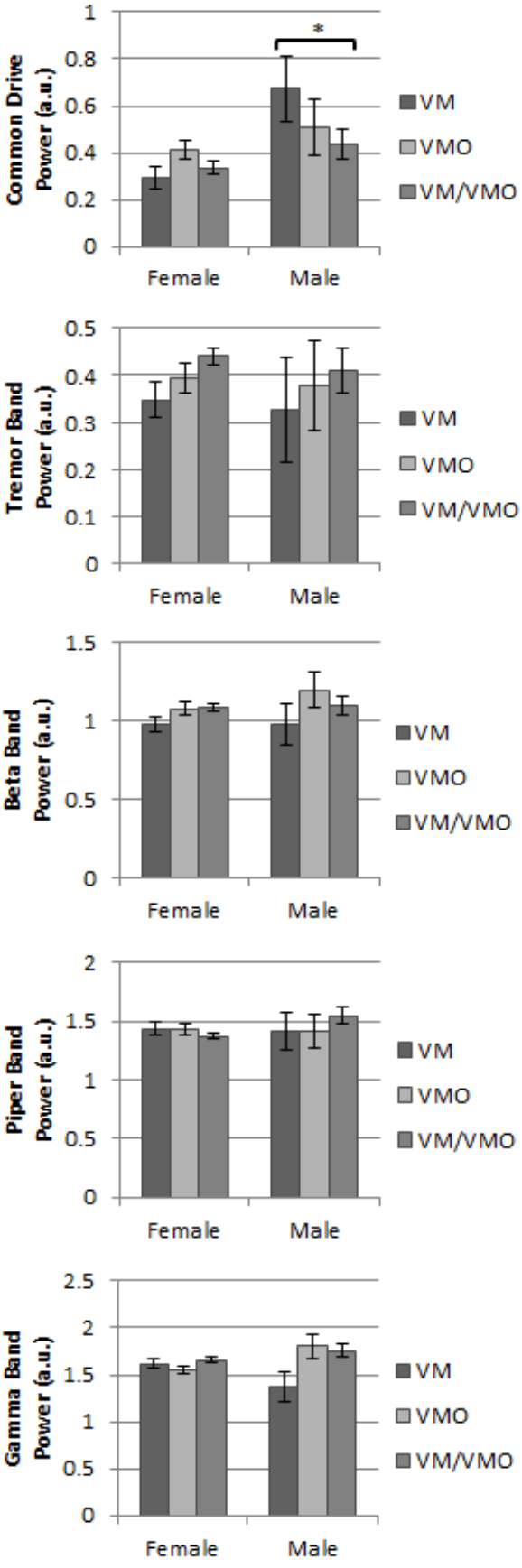


Figure 3.2. MU-EMG Coherence Power in Frequency Bands

Note: * indicates $p<0.05$

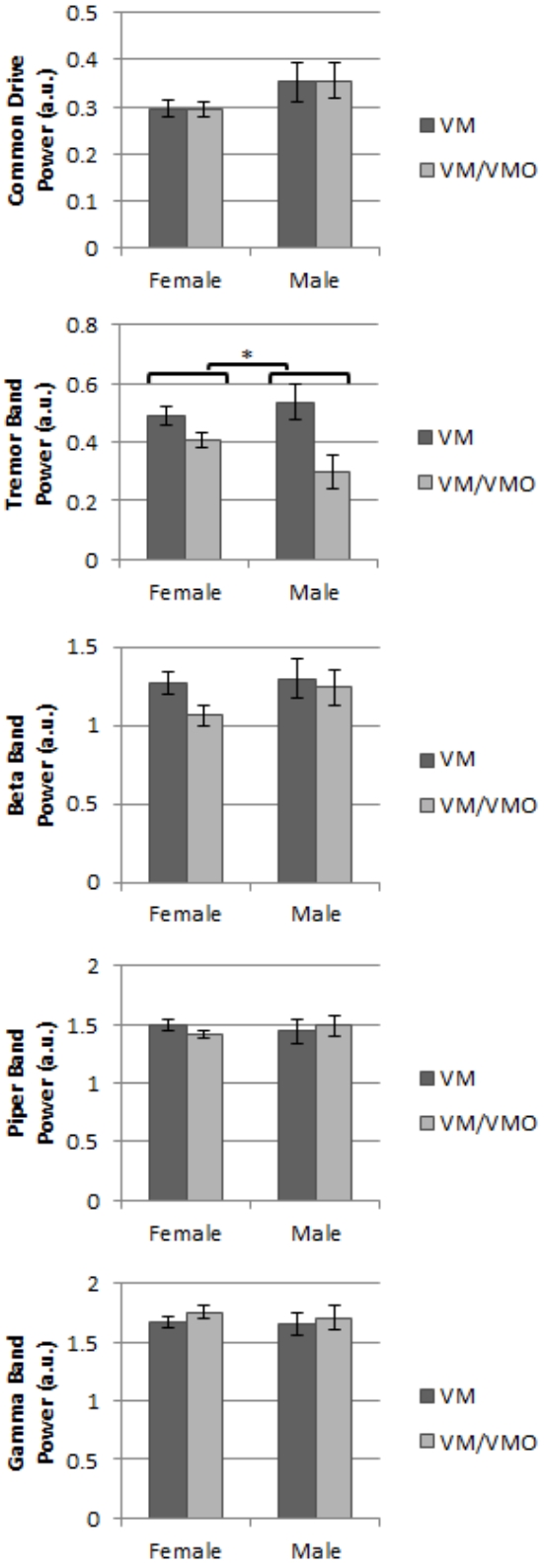


Figure 3.3. MU-MU Coherence Power in Frequency Bands across the Menstrual Cycle

Note: ▲= VMO; ■ = VM; ♦= VM/VMO

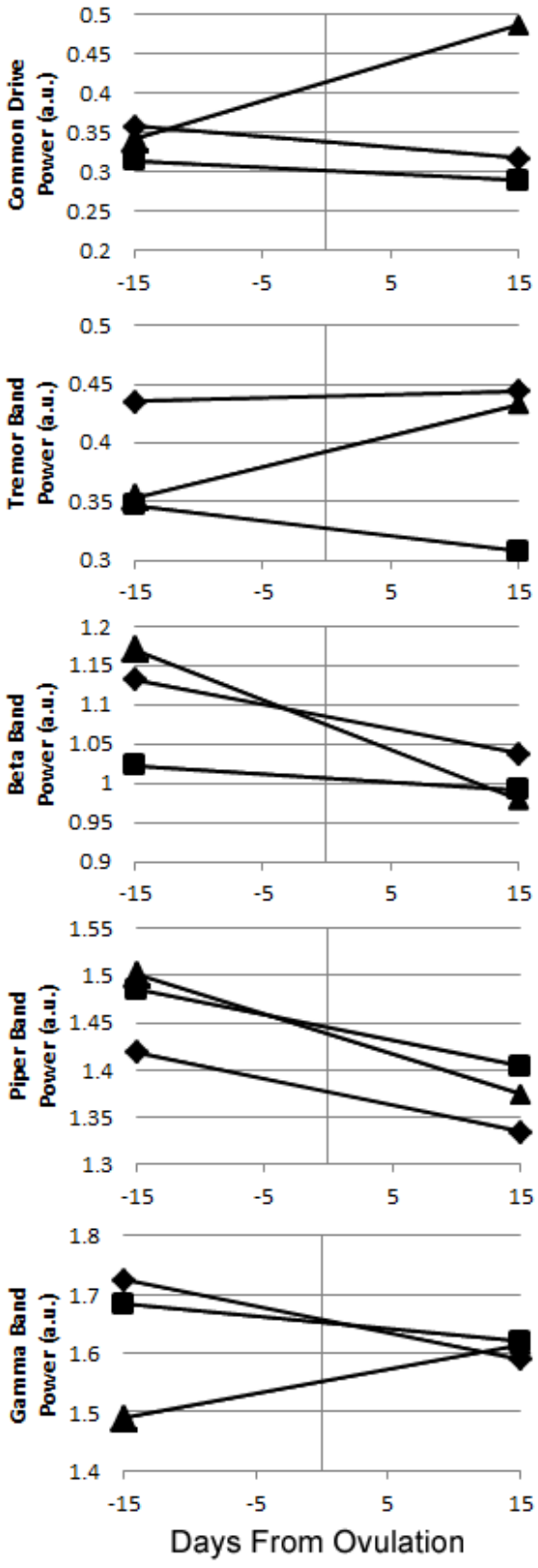
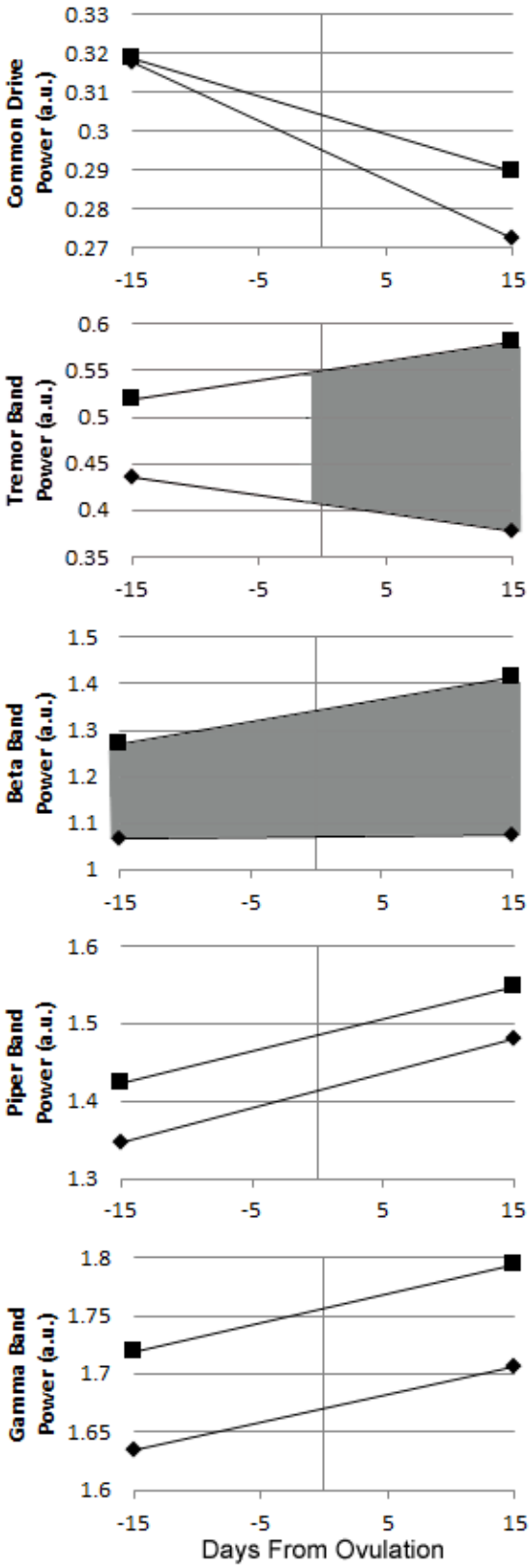


Figure 3.4. MU-EMG Coherence Power in Frequency Bands across the Menstrual Cycle

Note: Gray areas indicate significant difference regions. ■ = VM; ♦= VM/VMO



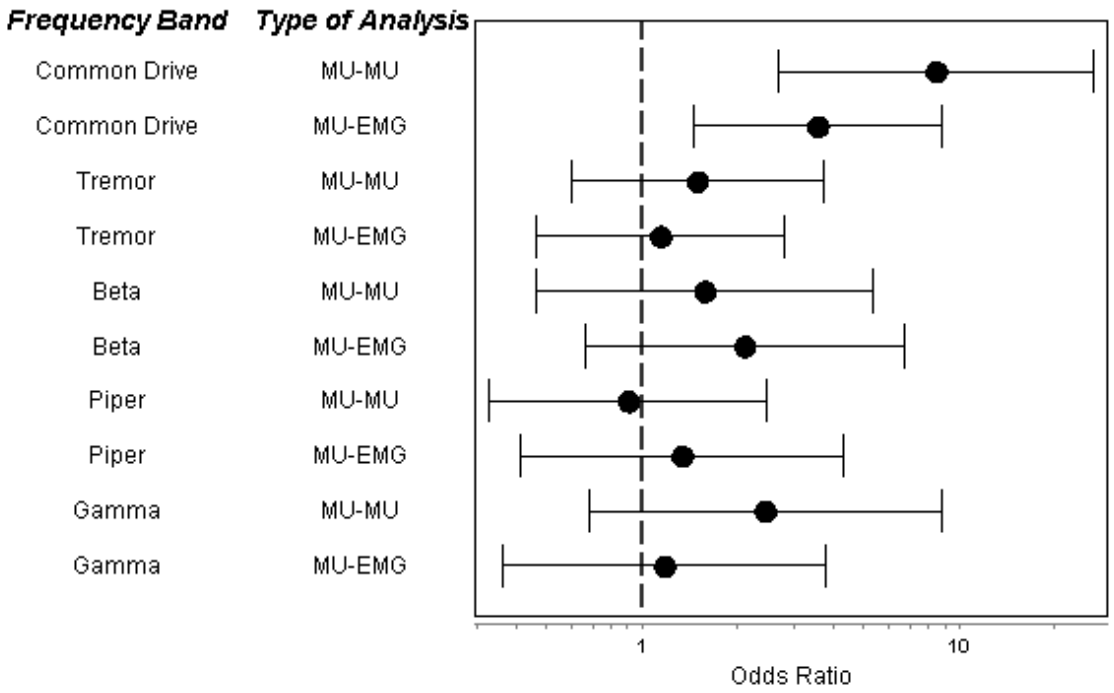


Figure 3.5. Forest Plot of Odds Ratio Estimates for Sex (±95% CI)

Note: The dashed line at 1 is the Female base model.

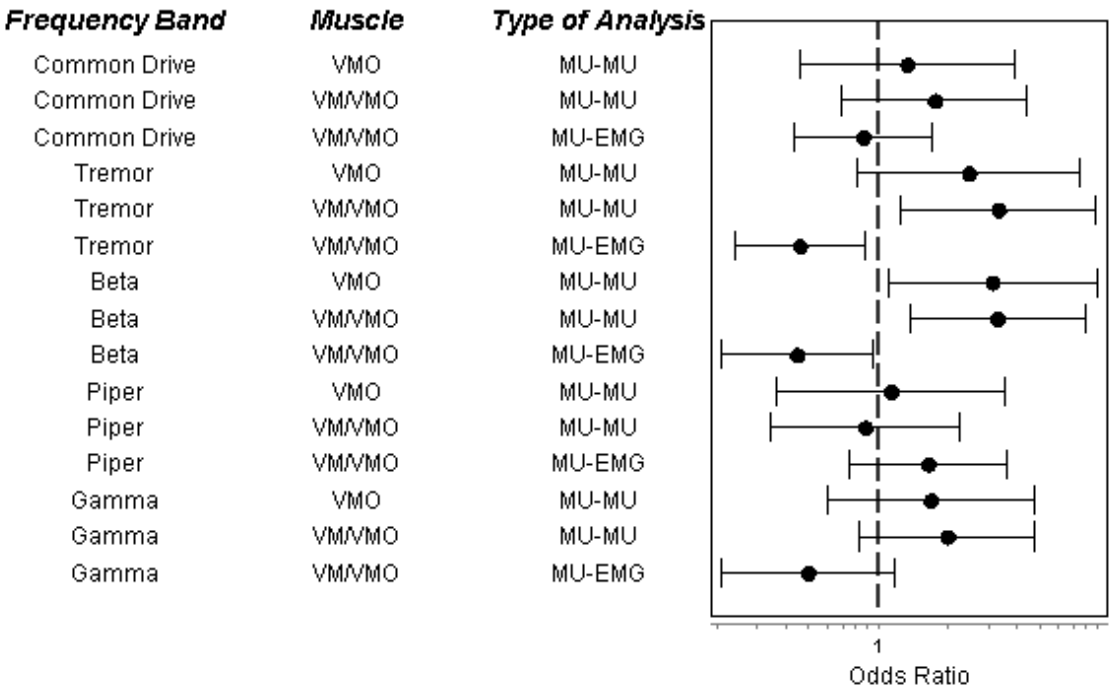


Figure 3.6. Forest Plot of Odds Ratio Estimates for Muscle Groups (±95% CI)

Note: The dashed line at 1 is the VM base model.

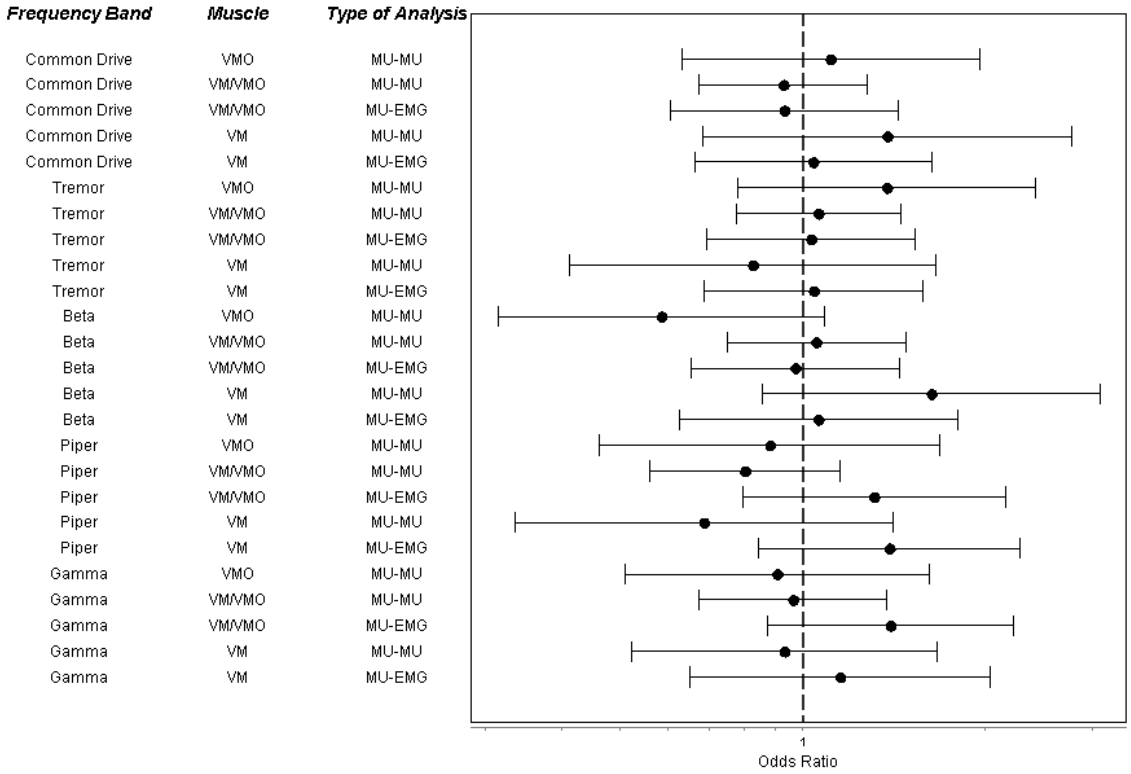


Figure 3.7. Forest Plot of Odds Ratio Estimates (±95% CI) across the Menstrual Cycle

Note: The dashed line at 1 is at ovulation day 0 with the odds ratio estimate at 7 days post ovulation.

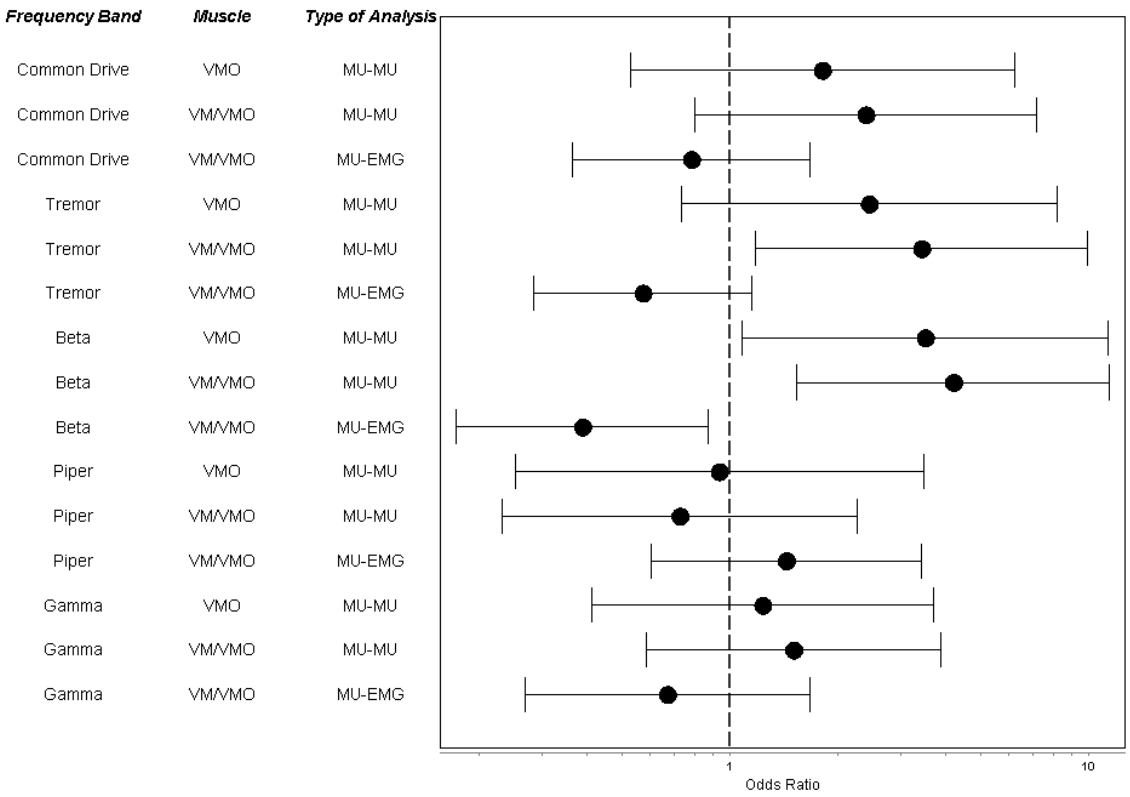


Figure 3.8. Forest Plot of Odds Ratio Estimates (±95% CI) for Muscle, Holding Menstrual Cycle Constant

Note: The dashed line at 1 is the VM base model.

[2]Chapter 4: Changes in Resting Heart Rate Variability across the Menstrual Cycle

ABSTRACT

Heart rate variability (HRV) is a non-invasive indicator of autonomic control. This study examines HRV changes across a normal menstrual cycle and proposes a novel piecewise function controlling for the effects of breathing on HRV spectral parameters. A resting ECG was collected from 13 women at five points in their menstrual cycle. Both heart rate and breathing rate increased across the cycle ($p<0.01$) while time-domain variability decreased ($p=0.04$). Use of the piecewise function for breathing rate in HRV spectral analysis was confirmed by a substantial increase in model goodness-of-fit. HRV spectral parameters, controlled for breathing with the piecewise function, confirm the decrease in variability is likely due to a parasympathetic withdrawal, since high frequency HRV decreases ($p=0.02$).

INTRODUCTION

The analysis of heart rate variability (HRV) is a commonly used non-invasive measure of autonomic cardiovascular control. Since the early work by Akselrod (1981), the oscillatory (frequency domain) components of HRV have been described in the high frequency (HF) band (0.15-0.40 Hz) and the low frequency (LF) band (0.04-0.15). At rest, time-domain HRV (SDNN) and HF power are metrics of parasympathetic tone

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[2] This study has been accepted for publication in Psychophysiology.
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RM Brothers contributed to experimental conception and design and critically revising of the manuscript.
AJ Tweedell contributed to data collection, analysis, data interpretation and critically revising of the manuscript.
AC Hackney contributed to experimental conception and design and critically revising of the manuscript.
L Griffin contributed to experimental conception and design and critically revising of the manuscript.

(Hayano et al., 1991). Early studies suggested that LF, particularly when normalized or in ratio with HF, was an indicator of sympathetic tone (Malliani et al., 1991; Pagani et al., 1997). Current literature presents a compelling argument that LF is primarily the product of phasic baroreceptor activity (Goldstein et al., 2011). Multiple techniques, including the "gold standard" Oxford technique, have indicated that LF is predominately modulated by the baroreceptor-cardiovagal gain (Rahman et al., 2011), though this has also been disputed (Reyes del Paso et al., 2013). Although the exact physiologic underpinnings of the LF band are still being delineated, the use of HRV as a measure has increased as a non-invasive way to gain insight into various physical and mental perturbations in both men and women.

Investigations using HRV in women may have unrecognized inconsistency if HRV cannot be considered stable across the menstrual cycle. An inconsistency in HRV readings across the menstrual cycle may lead to inappropriate conclusions based upon unstable baseline readings or a narrowed difference between baseline and physiologic ceiling. An increase in sympathetic drive or decrease in parasympathetic activity after ovulation is reasonable because studies have consistently shown an increase in metabolic rate in this period (Solomon et al., 1982; Webb, 1986; Bisdee et al., 1989), implicating luteal phase progesterone. Recently, several studies have investigated the effect of the menstrual cycle on HRV (Sato et al., 1995; Yildirir et al., 2002; Leicht et al., 2003; Bai et al., 2009; Mckinley et al., 2009). Previous research has reported a decrease in time-domain HRV in the luteal phase compared to the follicular phase (Mckinley et al., 2009) and frequency-domain changes, indicating decreases in parasympathetic and/or increases in sympathetic activity from the follicular to luteal phases (Sato et al., 1995; Yildirir et al., 2002; Bai et al., 2009). However, there has also been reported to be no change in frequency-domain HRV across the menstrual cycle (Leicht et al., 2003).

All previous studies examining HRV across the menstrual cycle have utilized only two or three testing sessions. Two or three testing points may be an over-simplification of the menstrual cycle, which exhibits a number of hormonal periodicities. Furthermore, the known increase in ventilation from the follicular to luteal phases (Slatkovska et al., 2006; Girija & Veeraiah, 2011) may account for the frequency-domain changes across the menstrual cycle, possibly indicating that the observed HRV changes across the menstrual cycle are artifactual in nature.

Breathing frequency substantially impacts HRV spectral parameters, whereas the time domain parameters are not affected by breathing frequency or tidal volume (Brown et al., 1993). Total spectral power decreases when breathing rate increases and when people breathe at rates between 7-10 breaths/minute, the respiratory sinus arrhythmia obscures the HF and LF divide, making interpretation of the autonomic properties difficult (Brown et al., 1993; Cammann & Michel, 2002; Beda et al., 2007). The respiratory sinus arrhythmia peak in the power spectrum is less prominent in spontaneous breathing than in metronome-controlled breathing at the same rate (Bloomfield et al., 2001). However, controlled metronome breathing can be a stressor unto itself (Patwardhan et al., 1995), potentially confounding the assessment of autonomic balance.

Therefore, the goal of this study was two-fold: 1) record HRV at five time points across the menstrual cycle to more effectively track the changes throughout the cycle and 2) use a novel piecewise ANCOVA function analysis to control for changes in breathing rate which may obscure actual changes in frequency-domain HRV. The piecewise function for breathing rates higher and lower than 10 breaths per minute allows for the unbiased examination of frequency-domain HRV by accounting for the effects of breathing rate without the addition of methodological stressors (i.e., controlled metronome breathing). We hypothesized that HRV variables would indicate less

parasympathetic control after ovulation and that the use of the piecewise function will contribute explanatory information when examining HRV across the menstrual cycle.

METHODS

Participants & Ethical Approval

Thirteen young eumenorrheic women (ages 20-31 years) participated in this study. All participants were free from cardiovascular, neurologic, endocrine or metabolic disorders, had a self-reported history of normal menstrual cycles, no previous history of pregnancy and were contraception naïve for at least 6 months prior to testing. Additional participant demographic information is provided in Table 4.1. All participants gave their informed consent in accordance with the Helsinki Declaration and all experimental procedures were approved by the University of Texas at Austin Institutional Review Board.

Determination of Study Visit Days and Ovulation

Data was collected from each participant at five points in the menstrual cycle, corresponding to the early follicular, late follicular, ovulatory, mid luteal and late luteal menstrual phases. The first point of data collection for each subject was randomized and resulted in a pseudo-counterbalanced design. Three participants started collection in early follicular, three in late follicular, two in ovulation, two in mid luteal and three in late luteal phase. The average cycle length for participants during data collection is in Table 4.1. All data was collected in the morning and time was standardized within each participant. Our use of the basal body temperature map (BBT) to determine menstrual phase has been previously reported (Tenan et al., 2013). The BBT has been used extensively for the determination of normal menstrual cycles and menstrual phases (Barrett & Marshall, 1969; Broverman et al., 1981; Prior et al., 1982; Bisdee et al., 1989;

Cassidy et al., 1994; Lebrun et al., 1995; Dunson et al., 2002; Viergiver & Pommerenke, 2013).

Procedurally, participants obtained their body temperature via oral thermometer for one month prior to data collection. Participants were instructed to take their oral temperature (BD Basal, Franklin Lakes, NJ) every morning before arising. Ovulation was operationally defined as the BBT nadir before the temperature rise in the luteal phase (de Mouzon et al., 1984). Previous research has shown the BBT to be 90% effective in determining ovulation when compared against luteinizing hormone determinations (Martinez et al., 1992). This biphasic response in the BBT is characteristic of a normal menstrual cycle in which ovulation occurs. If the temperature map from the first month was not clearly defined, the participant performed a second cycle map before admission to the data collection portion of the study. If the second temperature map was not well-defined, the participant did not enter data collection. The early follicular and late follicular phases are equally spaced before the three-day ovulatory phase; the mid luteal and late luteal phases are equally spaced after the ovulatory phase. The data collection point was equally spaced within the middle of each determined menstrual phase. The BBT was first assessed and then subsequently confirmed independently by two trained investigators. Two participants exhibited short luteal defects during the data collection portion of the study; another participant was anovulatory (no biphasic BBT) upon entry for their last data collection (ovulatory phase). Therefore, these three participants only completed four study visits. The data from subjects with a short luteal defect was included in the analysis as it has been shown that cycle length has little association with luteal progesterone or estradiol levels (Barrett et al., 2013). The ovulation date for the anovulatory participant was interpolated from the previous three months of normal BBT maps since menstrual cycle length was consistent. The follicular phase data for the

anovulatory participant was also entered into analysis as it has been shown that women occasionally presenting as anovulatory (oligomenorrhea) have estradiol concentrations within expected normal limits (Laven & Fauser, 2006). Finally, consistent overall cycle length (±2 days) and BBT was confirmed upon completion of data collection; mean intra-individual variation in menstrual cycle length of ±2.5 days is considered normal (Treloar et al., 1967). All participants collected at least 3 BBT maps over the course of the study.

Experimental Protocol

All data collection was performed in the Neuromuscular Physiology Laboratory at the University of Texas at Austin. Participants were instructed to not perform strenuous physical activity or ingest food containing large amounts of phytoestrogens 48 hours prior to testing. Additionally, the participants were instructed to avoid alcohol and caffeine for eight hours prior to the visit and any food or beverage, except water, two hours prior to their study visit. No further dietary restrictions or controls were applied to the participants.

The participants were seated in a padded adjustable chair with their relative hip and knee angles at 90 degrees. A seated position was used because this may more effectively represent daily human activity than the supine position. A standard 3-lead electrocardiogram (ECG) (Coulbourn Instruments, Allentown, PA), sampled at 1000 Hz (Micro 1401, Cambridge Electronics Design, Cambridge, UK), was used to obtain QRS wave timing. A piezoelectric chest harness (ADI Instruments, Sidney, Australia) was affixed inferior to the sternum to record the expansion and contraction of the chest cavity for determination of breathing rate.

After appropriate placement of the instruments was verified, the participant performed 20 minutes of seated rest. During the quiet rest period, the participant

remained seated with limited movement and was instructed not to speak. After completion of the rest period, 5 minutes of ECG and spontaneous breathing data was collected. The participants were instructed to face forward and keep their hands in their lap for the duration of the collection. No ectopic heart beats were observed.

Electrocardiogram & Breathing Data Reduction

The expansion-contraction of the chest cavity data obtained from the piezoelectric transducer was visually assessed and counted by one investigator. The breathing rate (breaths per minute) was determined by dividing the number of breaths by five (number of minutes data was collected).

The primary data reduction of the ECG signal was performed in ECGlab (de Carvalho et al., 2002). The ECG waveforms were manually assessed by one investigator to ensure correct identification of the QRS waveform. The R-R interval tachogram was also visually inspected for stationarity and aberrant classification. From R-R interval tachogram, the heart rate (HR) and SDNN were determined. The following frequency-domain metrics were obtained using the fast Fourier transform: total spectral power (TP, ≤0.4 Hz), LF (0.04-0.15 Hz), HF (0.15-0.4 Hz), and the ratio of LF to HF (LF/HF). The LF/HF ratio was assessed because it is commonly reported in the literature; however, the ratio is unlikely to represent sympathetic-parasympathetic balance since the LF band does not effectively convey information on sympathetic activity (Goldstein et al., 2011; Reyes del Paso et al., 2013).

Assessment of Normality

All data was first assessed to determine normality. Using a visual assessment of a bell-curve distribution, a Q-Q plot and the Shapiro-Wilk test for normality, only HR and breathing rate were determined to be normally distributed. All other variables were

determined to be normally distributed after performing a natural logarithmic transform (ln); therefore, all further statistical analysis and modeling was performed on the transformed data. A skewed HRV distribution has been reported previously in a large-scale study and the natural log transform has been applied to normalize the distribution (Tsuji et al., 1996). To aide in the interpretation of our findings and comparison with previous studies, we exponentiated the data in the presented figures back to their original units; however, all analysis and regression was performed on the transformed data.

Statistical Analysis

The primary interest in this study was the effect of menstrual cycle on HRV. To assess the changes in HRV across menstrual cycles of different lengths, the collection points were analyzed as the number of days from ovulation (BBT nadir) and total cycle length for each individual was included in the models as a covariate. All statistical analyses were performed in SAS 9.2 (Cary, NC).

Statistical Analysis: Time-Domain

To assess HR, breathing rate, and SDNN, a multilevel regression model was constructed using the maximum-likelihood estimation technique. This model used a first-order autoregressive covariance structure to account for the repeated-measures nature of the study design. Both the intercept and the slope of the variable of interest were allowed to vary (unstructured covariance structure) at the subject-level. This modeling approach allows each participant to have their own regression line which is then compiled to a model-wide regression (see Figures 4.1-4.3). The fit of this mixed effects model can be assessed via the concordance correlation coefficient (r_c) and interpreted similarly to the R^2 criterion (Vonesh et al., 1996). All models were fit with the linear time variable as well as a quadratic time variable; however, the addition of a quadratic term did not

increase model fit nor were the terms statistically significant. Therefore, the quadratic time variable was not included in the final analyses.

Statistical Analysis: Frequency-Domain

To assess TP, HF, LF and LF/HF, a multilevel regression model was constructed using the maximum-likelihood estimation technique. Because breathing rates higher and lower than 10 breaths per minute are known to impact spectral parameters of HRV (Brown et al., 1993), an a priori piecewise function was used as a covariate within the model. This piecewise function fits two slopes, one when the breathing rate is less than or equal to 10 breaths per minute and one when breathing rate is greater than 10 breaths per minute. This model used a first-order autoregressive covariance structure to account for the repeated-measures nature of the study design. Because model convergence could not be attained when both the slope and intercept varied at the subject-level, only the intercept was allowed to vary from the overall model. The goodness-of-fit for each model was assessed via r_c. Similar to the time-domain analysis, all models were originally fit with the linear time variable and a quadratic time variable, but the quadratic term did not increase model fit nor were the terms statistically significant. Therefore, the quadratic time variable was not included in further analyses. A reduced model, without the piecewise function, was fit as well as the full model for all variables to examine the utility of the function when analyzing spectral variables of HRV.

RESULTS

Time-Domain

HR and breaths per minute increased ($p=0.01$ and $p<0.01$, respectively) as a function of days from ovulation. Ten days after ovulation, HR increased 2.9 beats per minute (Figure 4.1) and breaths per minute increased by 0.8 breaths per minute (Figure

4.2). Variability (lnSDNN) decreased (p=0.02) across the menstrual cycle. Ten days after ovulation, lnSDNN decreases by 0.069 (exponentiated SDNN in Figure 4.3). The fit of all time-domain models as days from ovulation was good (see r_c in Figures 4.1-4.3).

Frequency-Domain

In the reduced model, without accounting for breathing rate, both lnTP ($p<0.01$) and lnLF (p=0.01) decreased across the menstrual cycle. No significant changes in lnHF (p=0.14) and lnLF/HF (p=0.31) were observed across the menstrual cycle. The fit of all reduced models was moderate-to-good (see r_c in Figure 4.4).

In the full model, accounting for breathing rate with a piecewise function, lnTP (p=0.02) and lnHF (p=0.03) decreased across the menstrual cycle; however, no significant change is observed in lnLF or lnLF/HF (p=0.12 and p=0.40, respectively). The fit of all full models was good (see r_c in Figure 4.5).

DISCUSSION

The results of this study demonstrate that HRV is higher prior to ovulation and that this variability decreases until the onset of new menses. HRV spectral parameters indicate that this effect is likely due to withdrawal of parasympathetic control of the autonomic nervous system. The changes are independent of changes in breathing rates, which were observed to increase across the menstrual cycle (i.e., follicular → luteal). HR also increased across the menstrual cycle, a likely result of the decreased parasympathetic control.

Evaluating the Piecewise Function for Spectral HRV Analysis

This study introduced a novel method of accounting for the effect of breathing rate on HRV spectral assessment. The piecewise function allowed different slopes of change when breathing rates were above and below 10 breaths per minute. Though HF is

commonly termed the "respiratory frequency", spontaneous breathing below a 10 breaths per minute average result in increased HRV signal power in the LF band (Cammann & Michel, 2002; Beda et al., 2007). Spontaneous breathing rates below 10 breaths per minute are not uncommon (Hoit & Lohmeier, 2000; Beda et al., 2007) and the rise in LF signal power appears to become exponential below 9 breaths per minute (Beda et al., 2007). The piecewise function introduced a negligible change in the fit and assessment of TP. However, in HF, LF and LF/HF, the fit of the model was discernibly increased and the resulting changes alter the interpretation of the data. Without use of the piecewise function, the spectral data results in the finding that no significant change in parasympathetic control (HF) is observed while the mixed parasympathetic/sympathetic/baroreflex frequency band (LF) decreases. An obviously erroneous interpretation of this result is that sympathetic activity decreases in later phases of the menstrual cycle without changes in parasympathetic activity. This interpretation is counterintuitive given the observed decrease in SDNN and increase in HR across the cycle.

The piecewise function for breathing rate maintains internal validity within the data. The results of the full ANCOVA model clearly indicate that the decrease in SDNN and increase in HR are largely mediated by decreases in parasympathetic control (HF) of the autonomic nervous system. The observed trend towards decreases in LF may be due to the nature of the frequency band encompassing parasympathetic, sympathetic and/or baroreflex activity since preliminary evidence suggest that baroreflex sensitivity is modified across the menstrual cycle (Tanaka et al., 2003), though this has been disputed (Cooke et al., 2002). As with all new analytic techniques, further validation of the piecewise function is needed to confirm that the HF and LF bands still represent their proposed autonomic analogues.

Decreases in Heart Rate Variability across the Menstrual Cycle

Our study is supported by previous reports indicating that HR increases (Mckinley et al., 2009; Girija & Veeraiah, 2011) and SDNN decreases when examining two data points in the follicular and luteal menstrual phases (Mckinley et al., 2009). However, other studies have failed to find changes in HR (Sato et al., 1995; Yildirir et al., 2002; Tousignant-Laflamme & Marchand, 2009) across the menstrual cycle and some show SDNN increases in later menstrual phases (Vallejo et al., 2005). The discrepancy with the previous literature may arise, in part, from previous studies not maintaining consistent data collection times (Yildirir et al., 2002; Vallejo et al., 2005), utilizing controlled metronome breathing (Patwardhan et al., 1995; Sato et al., 1995) and having a less stringent criteria for study participation (Tousignant-Laflamme & Marchand, 2009). The present study, examining 5 time points in the menstrual cycle, offers a more comprehensive view of HR and SDNN changes.

The results of this study indicate that the increase in HR and decrease in SDNN are mediated by a withdrawal of parasympathetic control of the autonomic nervous system, evidenced by a decrease in HF. Interestingly, previous research has been unable to consistently correlate sex hormone levels with changes in LF or HF (Leicht et al., 2003; Bai et al., 2009). Controlling for breathing rate with a metronome or failing to control for breathing rate at all may contribute to the failure to find changes in HRV spectral parameters across the menstrual cycle, an issue we have successfully resolved with our piecewise function. Nevertheless, the ability to find HRV changes across the menstrual cycle (Yildirir et al., 2002; Bai et al., 2009; Mckinley et al., 2009) but not in correlation with the sex hormones oscillating across the menstrual cycle is surprising. Two phenomena may contribute to this discrepancy: 1) the HRV changes observed across the menstrual cycle result from interactive effects of multiple sex hormones and 2) the

HRV effects observed may not occur in temporal continuity with observed sex hormone levels in the serum. The results of the present study should not be affected by either of these phenomena because the goal of this study was to characterize the changes across the cycle without regard for contribution of specific hormones.

Implications of Decreased Parasympathetic Activity across the Menstrual Cycle

The parasympathetic withdrawal observed later in the menstrual cycle corroborates previous research demonstrating increases in basal body temperature (Buxton & Atkinson, 1948; Lundy et al., 1974; Zuspan & Rao, 1974), increases in basal metabolic rate (Solomon et al., 1982; Webb, 1986) and increases in ventilation during luteal phases of the menstrual cycle (Slatkovska et al., 2006; Girija & Veeraiah, 2011). Progesterone appears to be the main driver for increases in ventilation in the luteal phases, although there is a notable secondary effect of estrogen as well (Regensteiner et al., 1989). Indeed, progesterone has a higher uptake in the hypothalamus than in other portions of the brain in rats (Seiki et al., 1968). A simple mechanism could be proposed whereby increased progesterone in the luteal phase causes parasympathetic withdrawal resulting in increases in metabolic rate, ventilation and body temperature; however, the main parasympathetic neurotransmitter, acetylcholine, has been shown to stimulate the secretion of estrogen and progesterone from human granulosa cells (Kornya et al., 2001; Bodis et al., 2002), an effect not seen by other catecholamines (Bodis et al., 2002). Obviously, the cause-and-effect interplay of the autonomic nervous system and the endocrine system is an intertwined ecosystem. As this interplay may substantially impact clinical research using HRV, further study is needed in this area.

Study Limitations

The results of this study cannot be generalized to postmenopausal or amenorrheic women as well as women taking hormonal birth control, lowering the population to which this particular study can be applied. However, the Centers for Disease Control reported that only 21.6% of reproductive-age women use hormonal birth control in the United States (Jones et al., 2012), indicating a substantial population benefit from research on eumenorrheic women. The time intensive nature of well-controlled studies across the menstrual cycle commonly result in a low sample size (Sato et al., 1995; Hirshoren et al., 2002; Leicht et al., 2003; Bai et al., 2009), and this study is no exception. Given the sample size, our inclusion of three participants exhibiting slight menstrual cycle deviations may also have a confounding effect. Further studies with larger samples and hormonal data are needed confirm the present findings. Our preliminary use of the piecewise function is promising, though further research should perform a complete validation of the method and seek to fully understand the impact on interpreting HRV results.

Conclusions

This study examined the changes in HRV across the menstrual cycle at five observational time points. In addition to collecting data at more points in the menstrual cycle than previous research, this study introduced and demonstrated the utility of a novel piecewise function to account for the effect of breathing rate on HRV spectral analysis. This study demonstrates that heart rate variability decreases across the menstrual cycle and that this decrease is mediated by parasympathetic withdrawal. Future research should continue to validate and refine the use of the piecewise function to remove the ventilatory effects from HRV analyses. Future studies examining changes in HRV across the menstrual cycle should utilize a larger study sample and assess various hormones and

sex hormone binding globulin using a data series time-lag commonly found in econometrics research. The time-lagged data series analysis may reveal that the changes in HRV across the cycle do result from a predictable temporal lag with hormone oscillations.

	Average	Minimum	Maximum
Height (cm)	166.2	152.4	177.8
Weight (kg)	66.5	37.6	104.3
BMI	23.9	16.2	36
Cycle Length (days)	29.5	25	32

Table 4.1. Subject Demographics

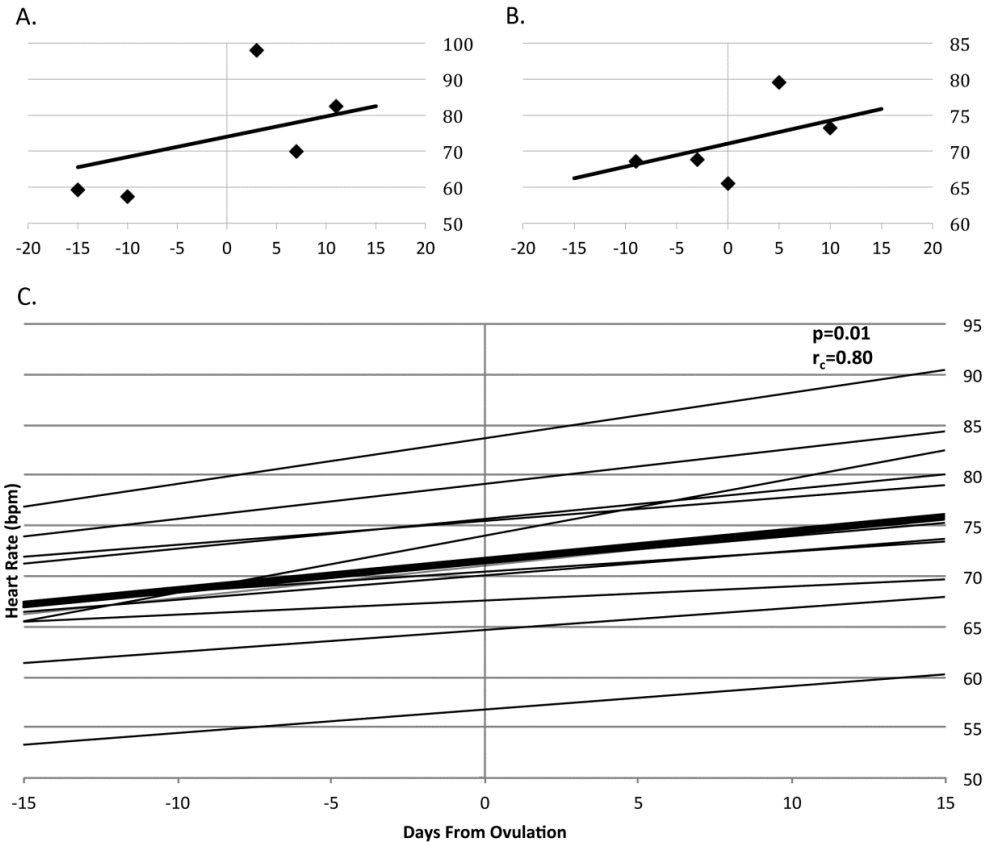

Figure 4.1. Heart Rate Changes across the Menstrual Cycle

Note: Time point zero is BBT nadir (ovulation). Plots A and B are data for two individuals with their subject-specific regression lines. Plot C depicts the individual lines for each subject (thin lines) and the model-wide regression line (thick line).

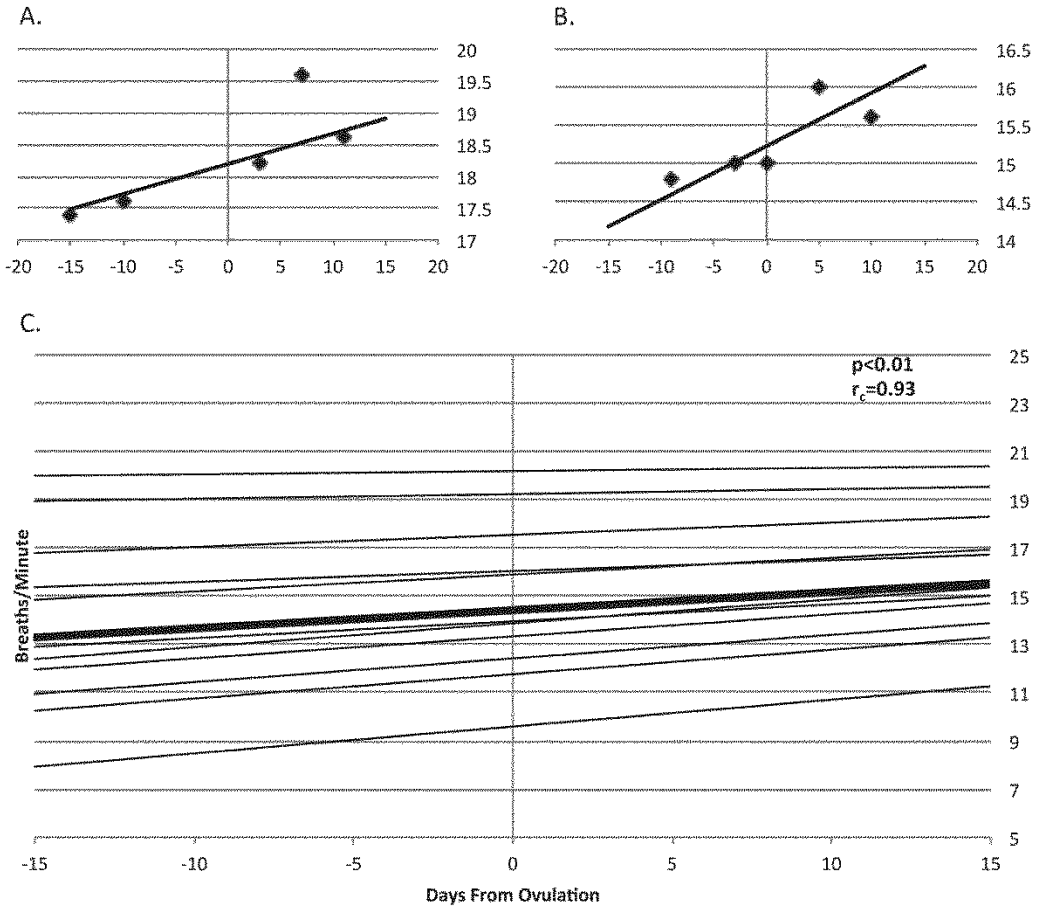

Figure 4.2. Breathing Rate Changes across the Menstrual Cycle

Note: Time point zero is BBT nadir (ovulation). Plots A and B are data for two individuals with their subject-specific regression lines. Plot C depicts the individual lines for each subject (thin lines) and the model-wide regression line (thick line).

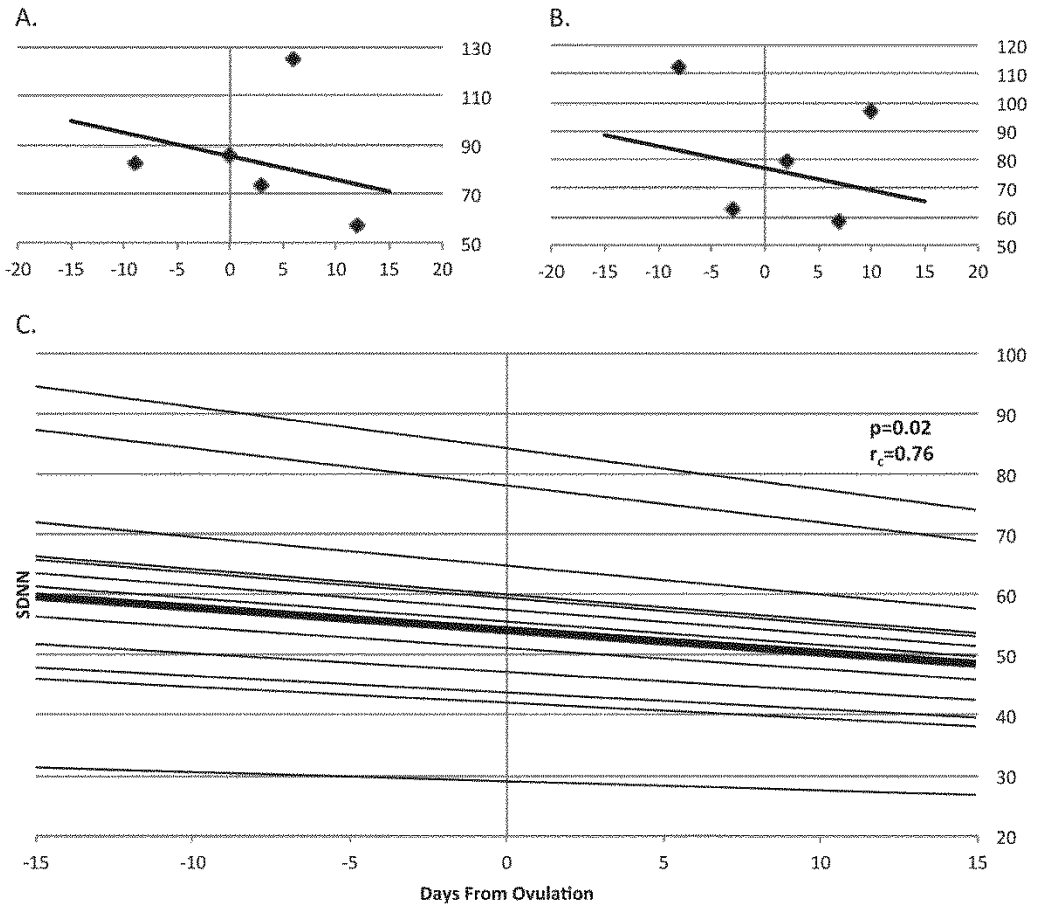

Figure 4.3. SDNN Changes across the Menstrual Cycle

Note: Time point zero is BBT nadir (ovulation). Plots A and B are data for two individuals with their subject-specific regression lines. Plot C depicts the individual lines for each subject (thin lines) and the model-wide regression line (thick line).

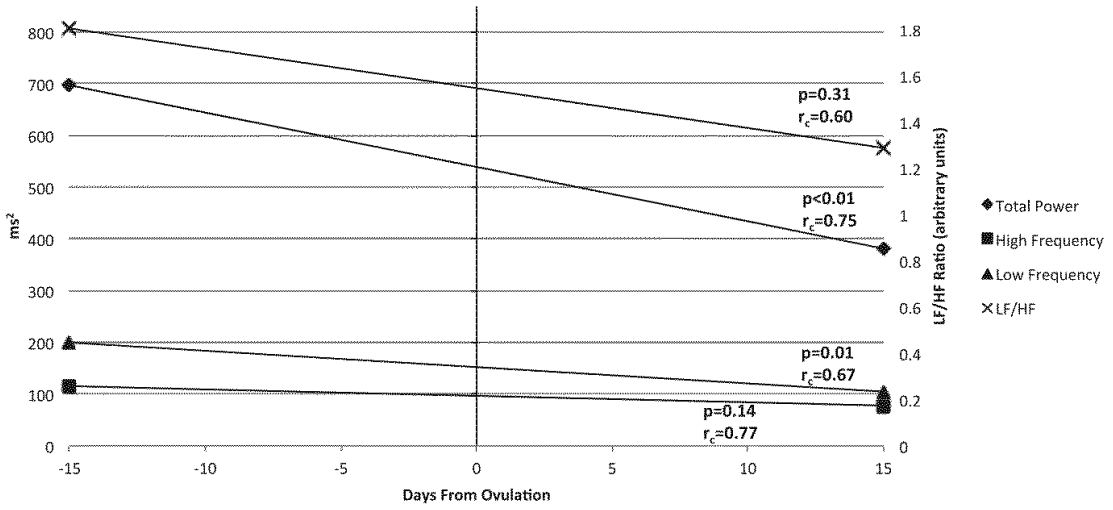

Figure 4.4. Reduced Model for Power Spectrum Changes across the Menstrual Cycle

Note: Time point zero is BBT nadir (ovulation). The left y-axis is the frequency content for total power, high frequency and low frequency spectrums. The right y-axis is the ratio of low frequency-to-high frequency spectrums.

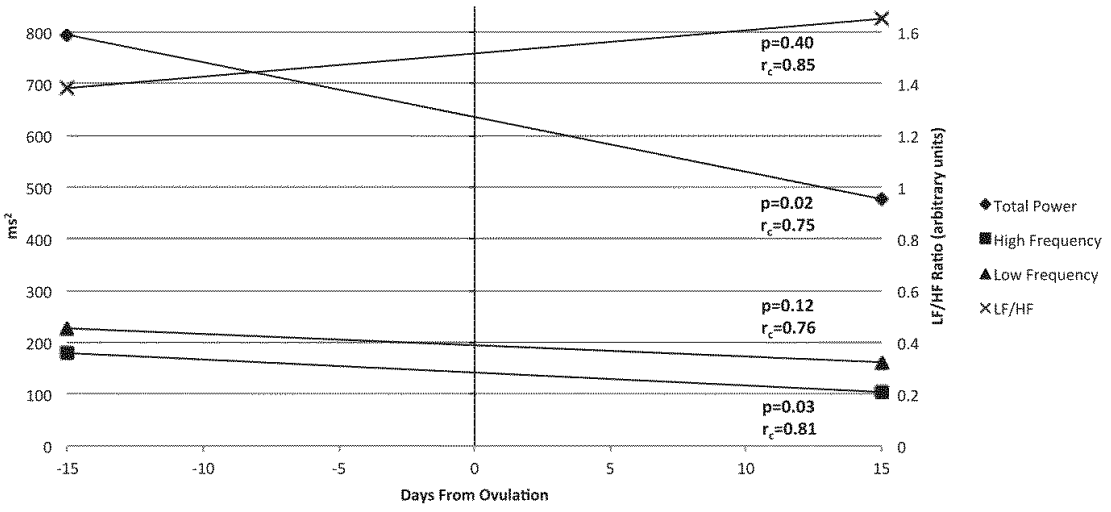

Figure 4.5. Full Model for Power Spectrum Changes across the Menstrual Cycle

Note: Model controls for breathing rate with a piecewise function. Time point zero is BBT nadir (ovulation). The left y-axis is the frequency content for total power, high frequency and low frequency spectrums. The right y-axis is the ratio of low frequency-to-high frequency spectrums.

Chapter 5: The Time and Frequency Domain Relationship between Electrocardiogram and Motor Unit Discharge: Effect of Sex and the Menstrual Cycle

ABSTRACT

Cardiovascular changes are often observed during exercise initiation. Previous research has shown that the motor and autonomic nervous systems can be activated in parallel. The goal of the present study was to determine the time and frequency domain relationship of the electrocardiogram QRS complex and motor unit (MU) discharge. The secondary goal was to characterize these relationships across the menstrual cycle. The electrocardiogram and single MU discharge trains from the vastus medialis oblique were collected during an isometric knee extension at 30% maximal force. Nine males participated in one visit. Nine eumenorrheic females participated in five study visits during the early follicular, late follicular, ovulatory, mid luteal and late luteal menstrual phases. Pooled coherence and cumulant density estimates were determined for each sex and menstrual phase. There was no uniform coherent pattern between the QRS timing and MU discharge in either sex or across the menstrual cycle. Both males and females had 20-25 millisecond lag between reference QRS waves and MU discharge. In females, this lag was found to persist in the early follicular phase. The late follicular, ovulatory and mid luteal phases had time lags between -5 to +5 milliseconds. The late luteal phase lag was 14 milliseconds. While no frequency domain relationship was apparent between QRS timing and MU discharge, there was a time domain relationship. Menstrual phases characterized by elevated levels of estrogen had a relationship centered around zero; whereas, phases with decreasing or low estrogen levels were centered around 14 and 25 milliseconds.

INTRODUCTION

Periodic oscillations in activity of both heartbeat intervals (Akselrod et al., 1981) and motor unit (MU) activity have been observed (Farmer et al., 1993; Conway et al., 1995). The entrainment of two neurons is indicative of a common central nervous system (CNS) origin (Farmer et al., 1993). This has been validated in the motor system by measuring EMG from active muscles and directly recording neurons at the primate motor cortex (Baker et al., 1997). Direct, high frequency simulation (>90 Hz) of the thalamus, subthalamic nucleus and substantia nigra all increase heart rate, mean arterial pressure and facilitate movement in awake patients with Parkinson's disease, demonstrating that deep brain stimulation can activate locomotion and the cardiovascular system in parallel (Thornton et al., 2002). While signal coherence has been used extensively in the motor control and EEG fields, the technique has never been applied to separate components of the nervous system to assess their entrainment for common CNS origin.

The congruence of two physiologic events can also be assessed in the time domain via various methods, such as the equivalent cross correlogram and cumulant density methods. Cardiovascular changes, determined by changes in ECG R-R interval, occur within 300 ms of exercise onset and shorten the first R-R interval an average of 30 ms (Williamson et al., 1995). Sympathetic nerve activity to the skin increases prior to or in concert with the onset of force generation during both actual force generation and simulated force generation (Leuenberger et al., 2003). These findings suggest that the motor and cardiovascular/autonomic systems can be activated in parallel and have a predictable temporal relationship.

Sex and the menstrual cycle may affect the neurological relationship of the cardiovascular and motor systems as our work has previously demonstrated that these systems are modified in isolation (Tenan et al., 2013; Tenan et al., 2014a). Furthermore,

estradiol has been shown to have powerful stimulatory effects on the nervous system via inhibiting the release of GABA in the striatum (Schultz et al., 2009). Therefore, it is possible that the neuronal origin enacting the parallel activation of the cardiovascular and motor centers may be modulated across the menstrual cycle and/or have an effect of sex.

The present study examined the temporal and spectral relationships between the electrocardiogram QRS complex and the discharge of MUs during an isometric knee extension task. Furthermore, the congruence of these two physiologic events were assessed between the sexes and at five points in the menstrual cycle.

METHODS

Participants & Ethical Approval

Nine males (24.8±5.3 years) and nine eumenorrheic women (24.7±4.5 years) participated in the study. Males participated in one study visit, all of which were conducted at 10:00 am. The females participated in five study visits at defined phases in the menstrual cycle: early follicular, late follicular, ovulation, mid luteal and late luteal. All females collected data in the morning. The time of data collection was standardized within each participant. Inclusion criteria for all participants were the absence of neurologic, cardiovascular, endocrine or metabolic disorders, previous leg surgery, immobilizations, arthritis, or chronic injury to the dominant leg. Additionally, the female participants must have been hormonal contraception naïve for at least 6 months prior to testing and have a history of clinically normal menstrual cycles. All participants gave their informed consent in accordance with the Helsinki Declaration and all experimental procedures were approved by the University of Texas at Austin Institutional Review Board.

Basal Body Temperature Determination of Menstrual Cycle Phase

Data was collected from the female study participants at five points corresponding to the early follicular, late follicular, ovulatory, mid luteal and late luteal menstrual phases. The first point of data collection for each subject was randomized and resulted in a pseudo-counterbalanced design with participants starting data collection in the following distribution: 2 early follicular, 2 late follicular, 1 ovulatory, 2 mid luteal and 2 late luteal. Our method of determining menstrual cycle phase via basal body temperature (BBT) in this cohort has been described previously (Tenan et al., 2013; Tenan et al., 2014a).

Briefly, participants obtained their BBT via oral thermometer for one month prior to data collection. They were instructed to take their oral temperature (BD Basal, Franklin Lakes, NJ) every morning before arising and record that temperature. A biphasic BBT is considered normal and ovulation is operationally defined as the nadir before the luteal phase temperature rise (de Mouzon et al., 1984). If the temperature map from the first month was not clearly defined, the participant performed a second cycle map before admission to the data collection portion of the study. If the second temperature map was not well-defined, the participant did not continue the study. The early follicular and late follicular phases are equally spaced before the three-day ovulatory phase; the mid luteal and late luteal phases are equally spaced after the ovulatory phase. The data collection point was equally spaced within the middle of each determined menstrual phase. The BBT was first assessed and then subsequently confirmed independently by two trained investigators. One participant did not have their electromyography data analyzed during their last study visit in the mid luteal phase because they exhibited a short luteal defect; however, the data from that participant's other trials was assessed because the late luteal trial was collected in the preceding cycle.

A second participant was found to be anovulatory, defined by a lack of biphasic response in the BBT, in their last study visit during the ovulatory phase; therefore, that participant only had four study visits because their data collection started in the mid luteal phase.

Experimental Protocol

All study visits were performed in the Neuromuscular Physiology Laboratory at the University of Texas at Austin. Participants were instructed to not perform strenuous physical activity or ingest food containing large amounts of phytoestrogens 48 hours prior to testing. Additionally, the participants were instructed to avoid alcohol and caffeine for eight hours prior to the visit and any food or beverage, except water, two hours prior to their study visit.

The experimental set up for both ECG (Tenan et al., 2014a) and MU (Tenan et al., 2013) data collection has been previously described. Briefly, participants were seated in an adjustable chair with the dominant hip and knee fixed at 90º. A standard 3-lead electrocardiogram (ECG) (Coulbourn Instruments, Allentown, PA), sampled at 1000 Hz (Micro 1401, Cambridge Electronics Design, Cambridge, UK), was used to obtain QRS wave timing. The waist and dominant thigh were immobilized with pads and straps. The participant performed 12 dynamic submaximal knee extensions without resistance before the dominant ankle was secured into a padded restraint attached to a strain gauge (Entran Sensors & Electronics, Fairfield, NJ). The participant performed three isometric maximal voluntary contractions (MVC) of the knee extensors, separated by 60 seconds of rest. The average of the three MVCs for that trial was used to ascertain the absolute force at which the participant would perform a sustained isometric knee extension during the test protocol.

After completing the MVCs, bipolar intramuscular insulated stainless steel fine-wire electrodes (0.002 mm diameter recording area, California Fine Wire Company, Grover Beach, CA) were inserted into the vastus medialis oblique muscle. The insertion point was immediately medial to the patella. A surface electrode ground was placed on the ipsilateral patella. The participant then practiced a steady force ramp up to 30% MVC. Target force feedback was visually provided by a screen directly in front of the participant at eye-level. The participant was asked to trace a line with their force output. For the data collection trial, the participant performed a force ramp up to 30% MVC and held that target force until they were instructed to terminate the exercise by the investigator. The exercise termination point was defined by either force oscillations greater than ±5% MVC or inability to maintain force output for three seconds. Data for fine-wire EMG and force were A/D converted (Micro 1401 Cambridge Electronic Design, Cambridge, England) and collected through Spike2 (version 5.21, Cambridge Electronic Design, Cambridge, England). Force and EMG were sampled at 1 kHz and 30 kHz, respectively.

Motor Unit and QRS Wave Data Reduction

All motor unit and QRS wave data reduction was performed in Spike2 (version 7.09a). Fine-wire EMG was band-pass filtered 100 Hz – 5 kHz with a 4th order Butterworth filter. The ECG waveform was unfiltered prior to QRS wave determination. Individual motor unit action potential trains and QRS waves were identified using Spike2's template matching algorithm. The classification was confirmed and edited by a singular investigator using Spike2's principal component analysis algorithm as well as a manual case analysis. Starting from the first classified motor unit action potential, a concurrently discharging motor unit train and QRS train were exported at 1000 Hz. The

motor unit train and QRS train discharged concurrently and continuously for 60 seconds in order to qualify for inclusion in the final analysis.

Time and Frequency Domain Analysis

All analyses were performed in Neurospec 2.0 (Halliday et al., 1995), a toolbox implemented in Matlab (version R214a, MathWorks, Natick, Massachusetts). Neurospec performs a coherence analysis for each individual simultaneous recording of MU/QRS train data. The number of segments analyzed for each study visit was fourteen 4.096 second segments. This results in a frequency resolution of 0.244 Hz. The large time segment was utilized to ensure that each segment included at least four QRS complexes for analysis. The individual coherence estimates are then combined or "pooled" to render a single representative estimate of the population (Amjad et al., 1997). The equal number of segments for each participant's study visit maintains equal weighting of the data. The cumulant density estimate is also calculated from the inverse Fourier transform of the cross-spectrum, resulting in a time domain metric of signal synchronization (Halliday et al., 1995) that can also be pooled (Amjad et al., 1997). The results are considered statistically significant when the coherence and/cumulant density estimate exceeds the 95% confidence limit calculated by Neurospec.

RESULTS

There was no uniformly coherent pattern between QRS waves and MU discharge in males, though statistically significant coherence can be observed around 5, 47 and 70-75 Hz (Figure 5.1A). When the female data is pooled across all menstrual cycles, there is a small magnitude oscillation at 90 Hz (Figure 5.1B). Similar to the pooled analyses of the sexes, there are periodic oscillations that exceed the 95% confidence limit in each menstrual phase; however, there does not appear to be any systematic change across the

menstrual cycle and the magnitude of all oscillations are below 0.08 in magnitude (Figure 5.2).

The cumulant density plots for both males and females indicate that there was a significant 20-25 millisecond (ms) lag between the reference QRS wave and the MU discharge train (Figure 5.3). When time-lags were assessed in each menstrual phase, a systematic change was evident (Figure 5.4). The early follicular phase had a 20-25 ms lag between QRS wave and the MU spike train (Figure 5.4A). The late follicular, ovulatory and mid luteal phases had lags which are evident between -5 and +5 ms (Figure 5.4 B-D). The late luteal phase lag was 14 ms (Figure 5.4E).

DISCUSSION

The results of the present study indicate that there are no clear and systematic coherent oscillations between the QRS complex and MU discharges. While statistically significant oscillations could be observed in each pooled analysis, they were all small in magnitude and did not appear to be repeatable or concentrated within any singular band. However, the time domain analysis demonstrated that time synchronization was observed in both sexes between 20-25 ms of lag, indicating that the QRS depolarization precedes the MU spike train depolarization by this time interval. The 20-25 ms lag was evident in the early follicular phase of the menstrual cycle, the phase in which female progesterone and estradiol levels are most similar to males. The late follicular, ovulatory and mid luteal phases, all characterized by elevated levels of estradiol, had temporal lags centered at -5 to +5 ms. The late luteal phase, characterized by falling levels of estradiol, had 14 ms time lag.

The failure to find clear and consistent oscillatory patterns between QRS and MU discharges is not entirely surprising. The QRS timing is a product of the autorhythmicity

of the sino-atrial node and modulation by sympathetic and parasympathetic inputs (Irisawa et al., 1993). This complex interaction of inputs and baseline depolarization activity may obscure any coherent oscillations with the motor system. Future studies should examine MU discharge trains in conjunction with sympathetic nerve activity or vagus nerve activity in isolation to determine conclusively if there is an apparent common neuronal origin of the two systems.

When assessing the temporal relationship of the QRS complex in relation to motor activity, the focus of previous research has been on determining the onset of change in relation to force output (Williamson et al., 1995; Liang et al., 2011). Liang et al. (2011) demonstrated that heart rate increases are observed 3 seconds prior to isometric dorsiflexion; however, their use of a QRS complex moving average (heart rate) without stating the precise algorithm used makes the interpretation and repeatability of their finding questionable. In a methodologically sound study, Williamson et al. (1995) examined the average R-R intervals at rest and contrasted these with the first 5 R-R intervals at the onset of volitional cycling. A shortening of the first R-R interval after cycling onset was noted within the first 300 ms and averaged a shortening of ~30 ms. While Williamson et al. (1995) did collect surface electromyography data, there was no apparent examination of this data in conjunction with R-R intervals prior to or in concert with exercise. To the knowledge of the author, the present study is the first to examine the time synchronization of the QRS complex intervals and the discharge of single motor units during a steady-state isometric exercise. Interestingly, the present study suggests that during isometric exercise, QRS complex alterations occur with the comparable MU discharge modification lagged by 20-25 ms. This finding cannot be easily explained in terms of a common origin and timing differential due to nerve conduction velocities. Indeed, peripheral sympathetic nerve conduction velocity ranges from 0.74 m/s to 1.69

m/s (Fagius & Wallin, 1980) and cardiac vagal nerve conduction velocity ranges between 5.6 m/s and 11.0 m/s (Kunze, 1972); whereas, peripheral motor nerve conduction velocity ranges from 36.5 m/s to 67.1 m/s (Thomas et al., 1959).

The change in observed latency timing between QRS and MU trains across the menstrual cycle also indicates that there is a probable effect of estradiol on the two systems interrelationship. Though the present study did not measure sex hormone levels, the phases which exhibit a QRS and MU train relationship centered near zero also have high levels of estradiol. The late luteal phase appears to represent a return of the time-lag to the level seen in the early follicular phase, due to decreasing levels of estradiol. Estradiol has been shown to enhance dopamine release in the striatum of female rats (Becker, 1990; Xiao et al., 2003), which should result in an increase in ascending motor input to the motor cortex. Estradiol has also been shown to decrease neural inhibition by binding ERα receptors on GABAergic neurons and inhibiting the release of GABA (Schultz et al., 2009). This combination of neurologic factors may potentially contribute to the decrease in time-lag between the QRS and MU trains when estradiol is elevated.

The differential time lag between the cardiovascular and motor systems across the menstrual cycle is interesting though hard to interpret without further studies. The change across the menstrual cycle appears systematic and physiological in nature; however, it is unknown if this interaction is a mere physiologic interest or if the modified interaction has an application to health and/or sports performance. Future research should explore this interaction further by directly assessing sympathetic nerve activity and motor unit discharge patterns during various exercise and pharmacologic perturbations.

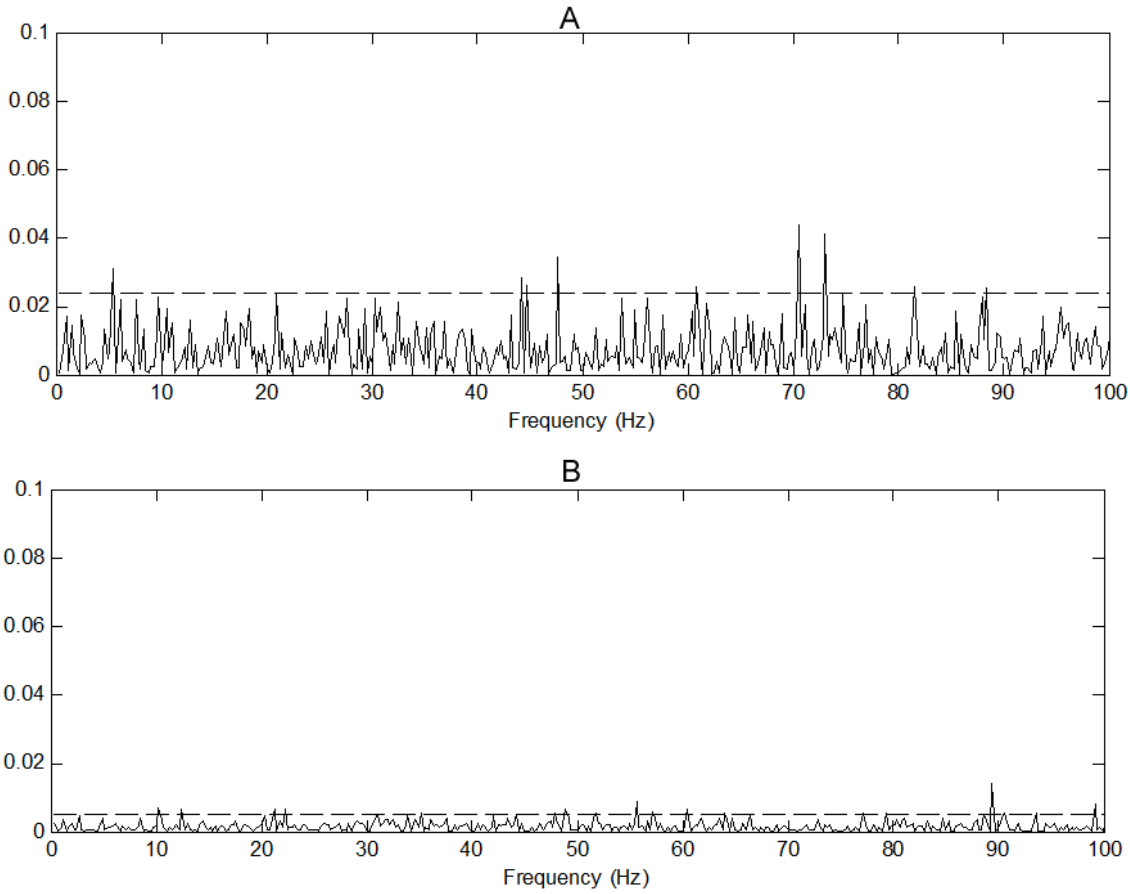

Figure 5.1. Pooled Coherence Estimates Plot for the Sexes

Note: Dashed line indicates 95% confidence limit. Subplot A is the male coherence plot. Subplot B is the female coherence plot.

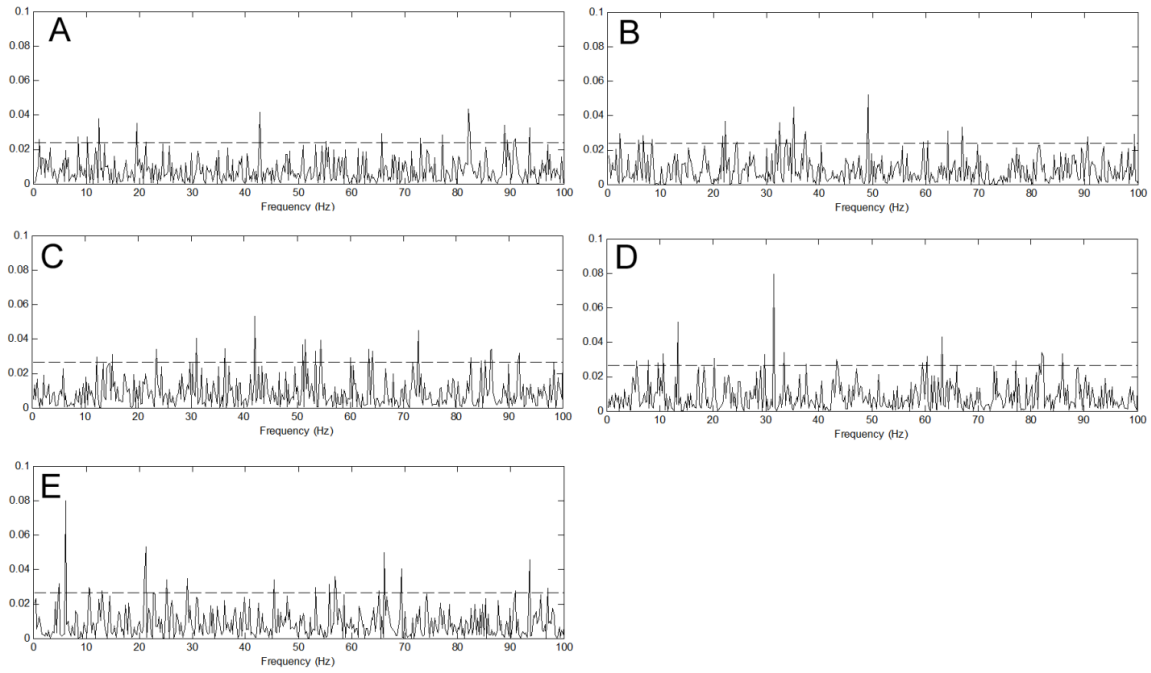

Figure 5.2. Pooled Coherence Estimate Plots for the Menstrual Phases

Note: The dashed line is the 95% confidence limit. The plots are early follicular (A), late follicular (B), ovulatory (C), mid luteal (D) and late luteal phases (E).

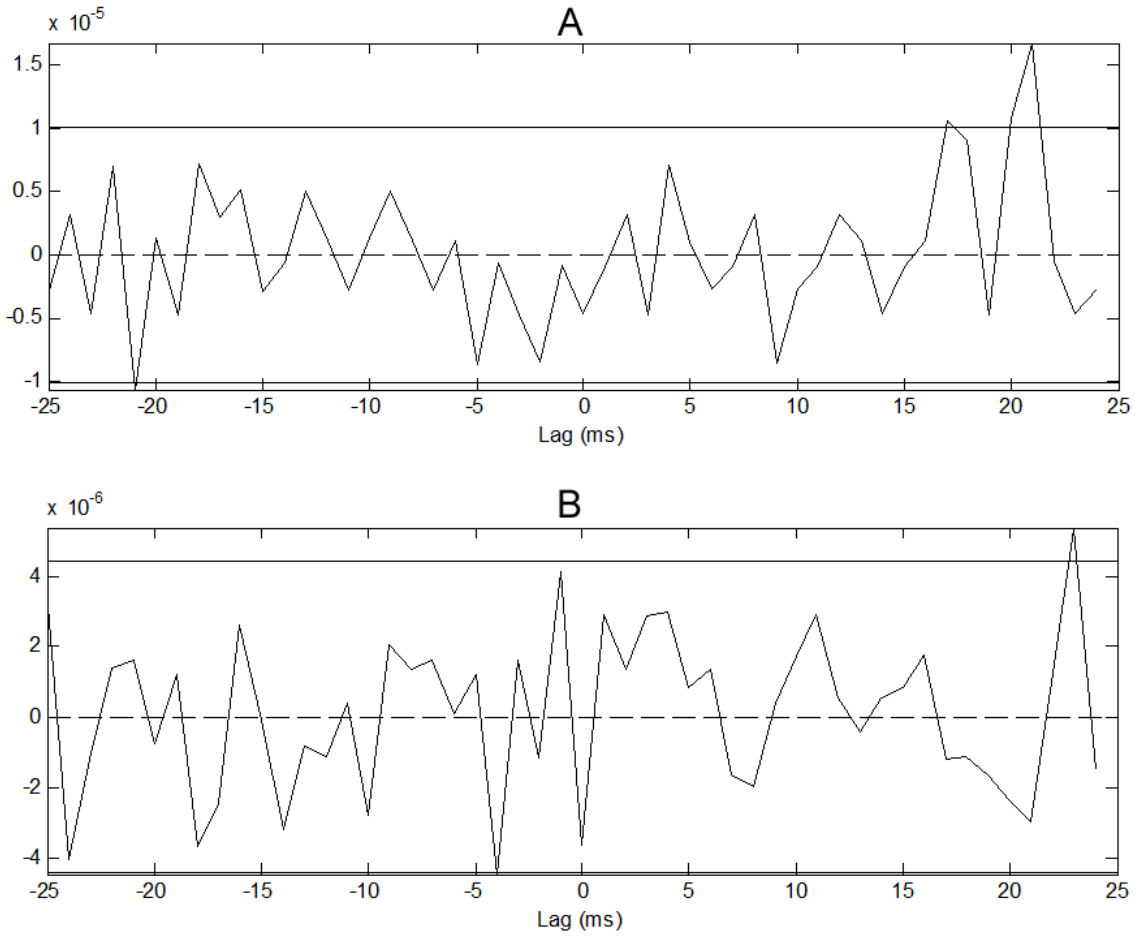

Figure 5.3. Pooled Cumulant Density Plot for the Sexes

Note: Dashed line indicates the null hypothesis. Solid bars indicate the 95% confidence limit. Subplot A is the male coherence plot. Subplot B is the female coherence plot.

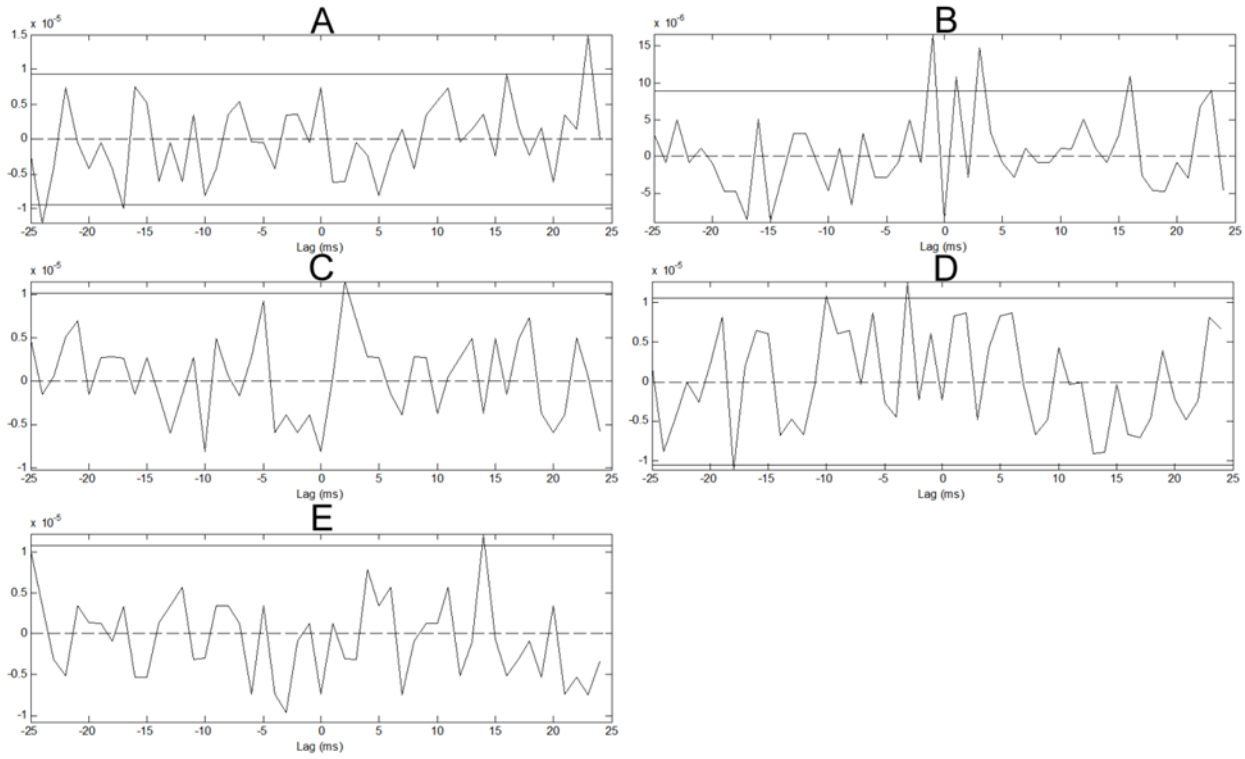

Figure 5.4. Pooled Cumulant Density Plots for the Menstrual Phases

Note: The dashed line is the null hypothesis. The solid line is the 95% confidence limit. The plots are early follicular (A), late follicular (B), ovulatory (C), mid luteal (D) and late luteal phases (E).

Chapter 6: Summary and Conclusions

Sex hormones have profound effects on nervous system development as well as short term nervous system communication. The aims of the present research were to investigate the sex and menstrual cycle differences as they occur in the motor and autonomic nervous systems to better understand how sex equitable research can be conducted and shed preliminary light onto observed sex differences in population-level data. The present dissertation demonstrated multiple instances of sexual dimorphism and autonomic and motor changes occurring across the menstrual cycle. These differences may, in part, explain discrepancies in rates of musculoskeletal injury, cardiovascular events and/or athletic performance between the sexes. Furthermore, scientific investigations which do not account for the sexual dimorphisms or fluctuations across the menstrual cycle are susceptible to inappropriate results and conclusions based upon poorly defined methodologies.

Previous studies have examined the effect of the menstrual cycle on involuntary force generation using TMS (Smith et al., 1999; Smith et al., 2002) and H-reflexes (Hoffman et al., 2008). Specific Aims 1 and 2 were the first to characterize voluntary movement generation in humans and how they differ between the sexes and across the menstrual cycle. Summation findings of Study 1 and 2 agree with involuntary studies that the excitability of the corticospinal tract is modified across the menstrual cycle (Smith et al., 1999; Smith et al., 2002) and that this excitability does not appear to be mediated at the spine (Hoffman et al., 2008). The Study 1 and 2 more effectively reflect the in vivo generation of moment which is the sum all ascending and descending cortical, subcortical and spinal inputs. Furthermore, the present study examined sex differences as well as a complicated musculoskeletal structure, the vastus medialis complex.

Our studies found that males have a common spinal origin and rate modulation for motor units innervating the vastus medialis and vastus medialis oblique, resulting in similar initial discharge rates at recruitment. Females are less likely to have this common spinal origin, allowing for the differential variations in initial discharge rate across the menstrual cycle. Irrespective of sex, the vastus medialis complex is a multifaceted skeletal structure. Control of sub-sections of the complex is modulated at the cortex and the distribution of this control has two primary areas distributed longitudinally. The geographic distribution of vastus medialis complex control is supported by macroscopic anatomical studies showing large portions of the population have distinct nerve trunks feeding distal and proximal vastus medialis sections (Smith et al., 2009) and that the distal subsection is innervated by a greater number of terminal nerve branches (Thiranagama, 1990).

Similar short-term effects of sex hormones affecting the motor system may influence changes in the autonomic system. Study 3 adds to the mounting scientific evidence that HRV is decreased in the luteal phases of the menstrual cycle (Yildirir et al., 2002; Bai et al., 2009; Mckinley et al., 2009) and that this reflects a decrease in parasympathetic activity. This change in the luteal phase may reflect the elevated uptake of progesterone within the hypothalamus (Seiki et al., 1968). Since stimulation of the hypothalamus creates a cardiovascular response as well as rhythmic leg movements (Smith et al., 1960), progesterone may have an effect on the co-activation of the autonomic and motor systems.

Study 4 examined the time and frequency domain relationship between motor unit discharges and the QRS complex of the electrocardiogram and how this relationship is modified by the menstrual cycle. Previous research examining the initiation of exercise and autonomic changes indicated that the co-activation of the systems was near-

simultaneous (Williamson et al., 1995; Leuenberger et al., 2003). The present study found that motor unit discharge lagged QRS depolarization by 20-25 milliseconds; moreover, this relationship is modified by the menstrual cycle. Menstrual phases characterized by high levels of estrogen had a relationship centered around zero milliseconds and low-estrogen phases are centered at 20-25 milliseconds, similar to males. Further studies are needed in order to understand the practical implications of this relationship and the apparent modification by the menstrual cycle.

There is growing acceptance in the field of applied physiology that sex and sex hormones affect a wide variety of non-reproductive systems. These dissertation studies further the understanding of sex differences and how sex hormone oscillations can have non-traditional effects on the motor and autonomic nervous systems.

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